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TITLE: 10th International Workshop on Campylobacter, Helicobacter & Related Organisms

PRINCIPAL INVESTIGATOR: Harry L. Mobley, Ph.D.

CONTRACTING ORGANIZATION: University of Maryland
Baltimore, Maryland 21201-1627

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Fort Detrick, Maryland 21702-5012

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**The 10th International Workshop on Campylobacter, Helicobacter & Related Organisms.**
September 12-16, 1999, Baltimore Maryland

The goals of this conference were to: 1) gather junior and senior investigators from around the world who specialize in areas encompassing all aspects of disease due to Campylobacter, Helicobacter, and Related organisms. These areas include public health, pathogenesis, renal and gastrointestinal pathophysiology, clinical management, food safety, and human and animal ecology; 2) present overviews and updates in these diverse areas by keynote speakers who are experts in their fields both inside the field and leading figures in pathogenesis; 3) present the latest results from investigators in these diverse areas in poster sessions and short invited talks; and 4) foster new collaborative multi disciplinary interactions to address pressing questions of prevention, pathogenesis, and treatment of disease due to Campylobacter and Helicobacter.

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PI - Signature Date

## Table of Contents

## INTRODUCTION

We proposed to host the 10th International Workshop on Campylobacter, Helicobacter & Related Organisms and over the past 18 years, our meetings have been held around the world with the world's leading authorities in the field. This biennial workshop, started in 1981, is an international forum dedicated to enhancing our knowledge of this fascinating group of bacterial pathogens. The 10th Workshop was held from September 12-16 in Baltimore, Maryland at the Renaissance Harborplace Hotel at the Inner Harbor. *Campylobacter jejuni* is now firmly established as the major cause of bacterial diarrhea in both developed and developing countries. *C. jejuni* is now known to also be one of the major causes of Guillain-Barre Syndrome and is attracting intense investigation worldwide. The genome sequence of *C. jejuni* wii be first discussed at this meeting. At the 2nd workshop in Brussels in 1983, *Helicobacter pylori* (then named *Campylobacter pylori*) was introduced to the world and the microbiological revolution of gastroduodenal disease began. The association of *H. pylori* with gastritis, peptic ulcers and gastric carcinoma is now universally recognized. An understanding of the disease process involving *H. pylori* and hopefully, the means to prevent or control it appears to be within reach as a great many scientists and clinicians are striving towards this end. The workshop included sessions on diagnostics, epidemiology, genetics, pathogenesis, food safety, antimicrobial therapy and testing, new and emerging pathogens, and Guillain-Barre syndrome. These international workshops provide the ideal opportunity for clinicians and scientists from a variety of disciplines to exchange information, formulate research strategies and update themselves on the latest information on these microorganisms of extreme medical importance. At least 450 specialists attended the meeting. This was the first such meeting to be held in the United States. The site of the meeting was Baltimore, Maryland, a location convenient for large numbers of attendees from North America and Europe.

## BODY

The meeting was successfully held and the **results of the deliberations are included on the accompanying CD-ROM proceedings of the meeting** (see attached CD-ROM). The support of the US Army is acknowleded in both the CD-ROM and in the final program, which is also attached. The results of the 18 workshops are individually listed as PowerPoint presentations on the CD-ROM.

## KEY RESEARCH ACCOMPLISHMENTS

**Problems to be clarified and potential scientific developments as a consequence of the meeting**

1. Guillain-Barre Syndrome - Role of autoimmunity in *Campylobacter jejuni*-mediated onset of neurological disease was discussed

2. Comparative genomics- The release of the *Campylobacter jejuni* genome sequence was unveiled. Candidate virulence determinants for this organism were discussed. The genome sequence of a second strain of *Helicobacter pylori* was discussed both in relation to the TIGR

sequence now available but also the *C. jejuni* sequence. New methods for sequencing genomes and analysis were also discussed.

3. Food Safety - In an area (the DelMarVa peninsula) that harbors at any given time 500 million chickens, strategies were developed to assess the effects of Campylobacter colonization on the safety of the poultry supply.

4. Spread of antibiotic resistance - Epidemiology studies were reported on the incidence of antibiotic resistance (e.g., clarythromycin and metronidazole resistance in *H. pylori*) in the United States and other countries.

5. New mechanisms of pathogenesis for *H. pylori* infection of the gastric mucosa of humans was discussed. Progress made using animal models of infection (primates, gnotobiotic piglets, and mice) was summarized by respective experts.

6. Progress on Helicobacter vaccines was reported. Multidisciplinary discussions assisted in the design of new vaccine trials.

7. Clarification of the significance of genome diversity. Both *Campylobacter* and *Helicobacter spp.* demonstrate tremendous DNA sequence variability between strain of the same species. The relevance of these differences were examined by experts in population genetics and molecular biologists.

**The results of these discussions are included on the accompanying CD-ROM.**

## REPORTABLE OUTCOMES

Mobley, H.L.T., I. Nachamkin, and D. McGee. 2000. Proceedings, 10th International Workshop on Campylobacter, Helicobacter, and Related Organisms. Proceedings on CD-ROM.

This document is attached to the report.

## CONCLUSIONS

Funding from the USAMRMC contributed towards the successful holding of the *10th International Workshop on Campylobacter, Helicobacter, and Related Organisms*. 18 workshops were held along with plenary sessions and poster sessions. The abstracts, the program, the summaries of workshops, and conference photographs are found on the accompanying CD.

## REFERENCES

Not applicable

## FINAL REPORTS

The final program and the CD-ROM proceedings are included with the report


BALTIMORE, MARYLAND • SEPTEMBER 12-16, 1999

# Abstracts and Final Program of the

# 10th International Workshop on *Campylobacter, Helicobacter* and Related Organisms

Baltimore, Maryland
September 12-16, 1999

**Compiled and edited by**

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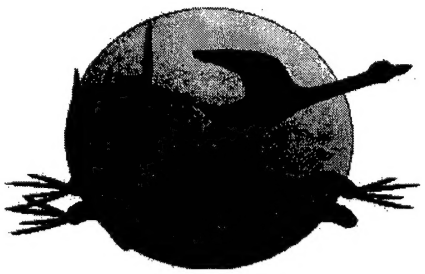

The Organizing Committee of the 10th International Workshop on CHRO would like to thank the following for their generous sponsorship:

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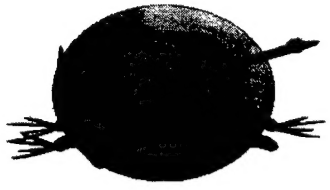

# 10th International Workshop on Campylobacter, Helicobacter, & Related Organisms

***September 1999***

***Baltimore, MD USA***

September 12, 1999

Dear Colleagues,

On behalf of the Local Organizing and Scientific Program Committees and the Board of International Advisors, I would like to welcome you to the *10th International Workshop on Campylobacter, Helicobacter & Related Organisms.*

This meeting, at the Inner Harbor of Baltimore, is being held for the first time in the United States. It has been two years since our last convening in CapeTown, South Africa and there have been many exciting developments including the completion of the genome sequences of *Campylobacter jejuni* and a second strain of *Helicobacter pylori.*

This week, in our Plenary Sessions, we will learn about new microbiological and immunological concepts from internationally recognized experts. Eighteen Workshops have been organized and formatted expressly to stimulate discussion and debate within our various subdisciplines. Attendees will present over 300 posters and 10 outstanding abstracts from Junior Investigators will be presented as oral presentations.

When our work is done for the day, join us in the evenings for our Opening Reception at the Renaissance Harborplace Hotel, Receptions at the Peabody Conservatory Library and National Aquarium, and our Closing Banquet. You may also choose to join in the traditional *Helicobacter* vs. *Campylobacter* Football (Soccer) match. In your spare time, wander around the Inner Harbor and the see the sites.

We're glad you're here. Please enjoy yourself.

Sincerely,

Harry Mobley

Harry L.T. Mobley, Ph.D.

**Organizer**
*Harry Mobley*

**Local Organizing Committee**
*David McGee*
*Irv Nachamkin*

**Scientific Committee**
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*Martin Blaser*
*Patricia Guerry*
*Harry Mobley*
*Irv Nachamkin*
*Carol Pickett*
*Norm Stern*
*Trevor Trust*

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*Jean-Paul Butzler*
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*Albert Lastovica*
*Adrian Lee*
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*Anthony Moran*
*Diane Newell*
*Guillermo Ruiz-Palacios*
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*Sebastian Suerbaum*
*Diane Taylor*

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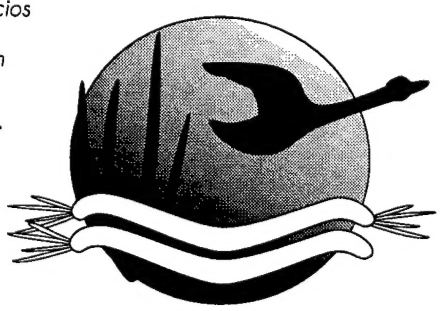

# 10th International Workshop on CHRO
# Committees and Acknowledgments

***Local Organizing Committee***

Harry Mobley, Chair
Irv Nachamkin
David McGee

***Scientific Committee***

Doug Berg
Martin Blaser
Patricia Guerry
Harry Mobley
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Norm Stern
Trevor Trust

***Meeting Coordinators***

Rick Deller
Melissa Greenhouse
Meeting Solutions

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Albert Lastovica
Adrian Lee
Francis Megraud
Anthony Moran
Diane Newell
Guillermo Ruiz-Palacios
Charles Penn
Sebastian Suerbaum
Diane Taylor

The web site for the meeting (www.med.upenn.edu/~campy10) was designed by Chris Coker, Department of Microbiology & Immunology, University of Maryland, Baltimore (Laboratory of Harry Mobley). The web site was installed, maintained, and frequently updated by Irving Nachamkin. This effort is greatly appreciated.

Thanks to members of the Local Organizing Committee, Scientific Committee, and International Advisors for reviewing abstracts, providing program suggestions, and securing sponsorship. The meeting could not take place without these efforts.

Special thanks to members of the Mobley lab, especially David McGee for willingly undertaking diverse tasks before and during the meeting.

# Poster Placement Information

## When and where do I put up my poster?

1. Look up the name of the presenting author and abstract title in the back of the Program Book and find the abstract number (example, CD12). This information is also in your registration bag and is posted near the registration area and the poster area.

2. Look on the page in your Program Book labeled "Poster Sessions at 10th CHRO" to see whether your abstract designation (example: CD) is in Session I or Session II.

3. Look at the footnote below your session (I or II) to determine when you may put up your poster, stand in front of your poster, and take down your poster.

4. To find the location of the board where your poster will be displayed, look at the Poster Map marked "Poster Session I" or "Poster Session II." You will find your abstract number at your designated location.

5. On the board itself, will be your abstract number and name.

6. Pins will be available to secure your poster.

## Your abstract number tells you which poster session you are in.

The first two letters of your abstract number designate your poster session topic according to the abbreviations below:

| | |
|---|---|
| CA or HA | Antimicrobial Agents |
| CB | Guillain-Barré Syndrome |
| CD or RD | Campylobacter Diagnostics and Clinical |
| CE or RE | Campylobacter Epidemiology and Subtyping |
| CF, HF, or RF | Food Safety |
| CG or RG | Campylobacter Genomics |
| CP | Campylobacter Pathogenesis and Animal Models |
| CV | Campylobacter Immunology and Vaccines |
| CX or HX | Population Genetics |
| HD | Helicobacter Diagnostics and Clinical |
| HE | Helicobacter Epidemiology |
| HG | Helicobacter Genomics |
| HP | Helicobacter Pathogenesis and Animal Models |
| HV | Helicobacter Immunology and Vaccines |

# Poster Sessions at 10th CHRO

## Poster Topics by Day / Times for Putting up and Taking down

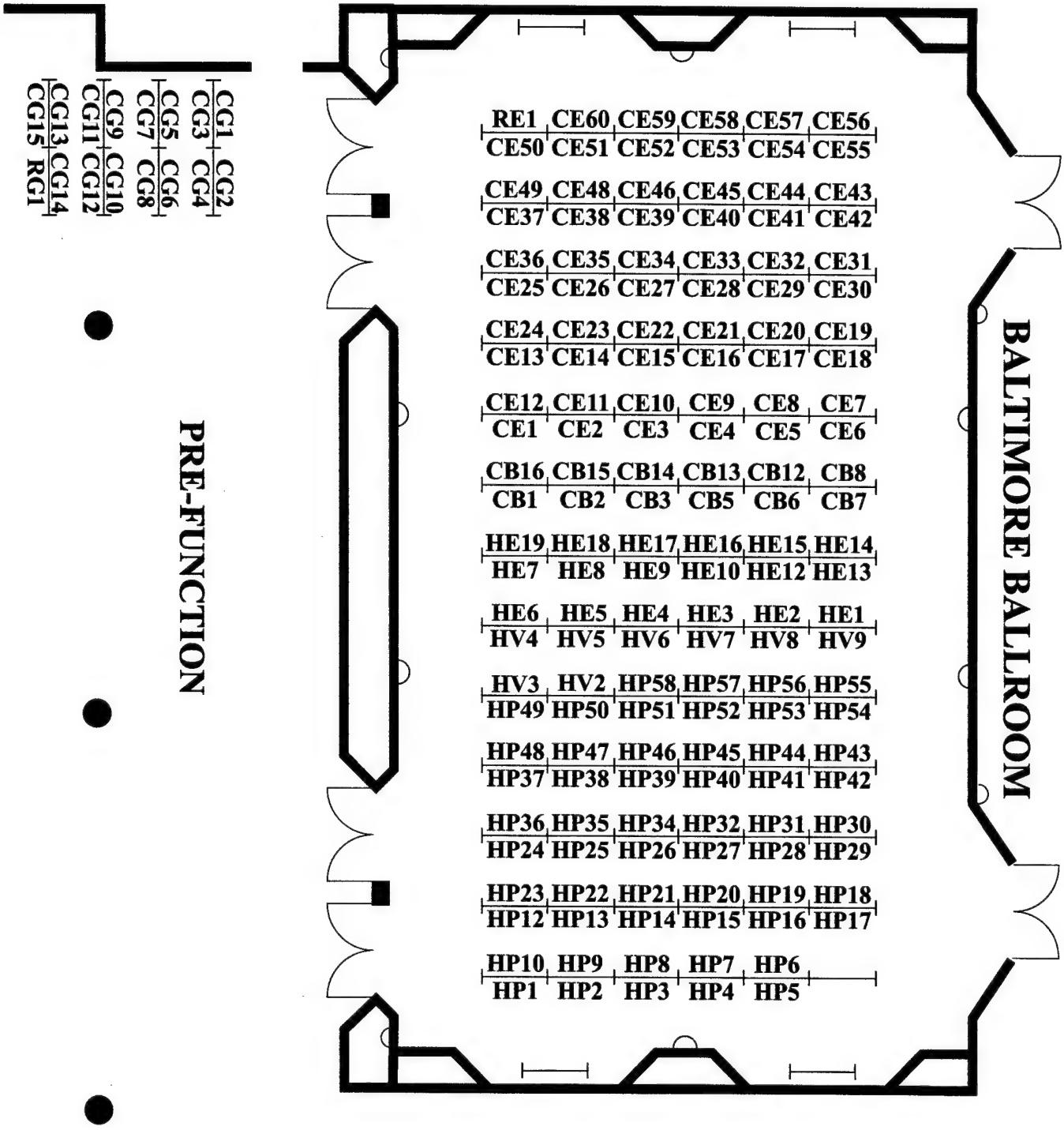
| Session I* | | | | Session II** | | | |
|---|---|---|---|---|---|---|---|
| Stand by poster on Monday 11:30am-1:30pm | | Stand by poster on Tuesday 11:30am-1:30pm | | Stand by poster on Wednesday 11:30am-1:30pm | | Stand by poster on Thursday 8:30-10:30am | |
| Campy Epidemiology and Subtyping | CE<br>RE | Helico Pathogenesis/Animal Models | HP | Campy Food Safety | CF<br>RF<br>HF | Helico & Campy Antimicrobial Agents | HA<br>CA |
| Helico Immunology/ Vaccines | HV | Campy Genomics | CG<br>RG | Campy Pathogenesis/Animal Models | CP | Campy Immunology/ Vaccines | CV |
| Helico Epidemiology | HE | Campy Guillain-Barre Syndrome | CB | Helico & Campy Population Genetics | HX<br>CX | Helico Diagnostics/Clinical | HD |
| | | | | Helico Genomics | HG | Campy Diagnostics/Clinical | CD<br>RD |

***All posters for Session I may be put up Sunday after 4 PM, but must be put up by Monday at 8:30 AM**
**Posters *must be removed* by 6:30 PM on Tuesday.**

****All posters for Session II may be put up after 6:30 PM on Tuesday, but must be put up by Wednesday at 8:30 AM.**
**Posters *must be removed* by 12:00 PM on Thursday**

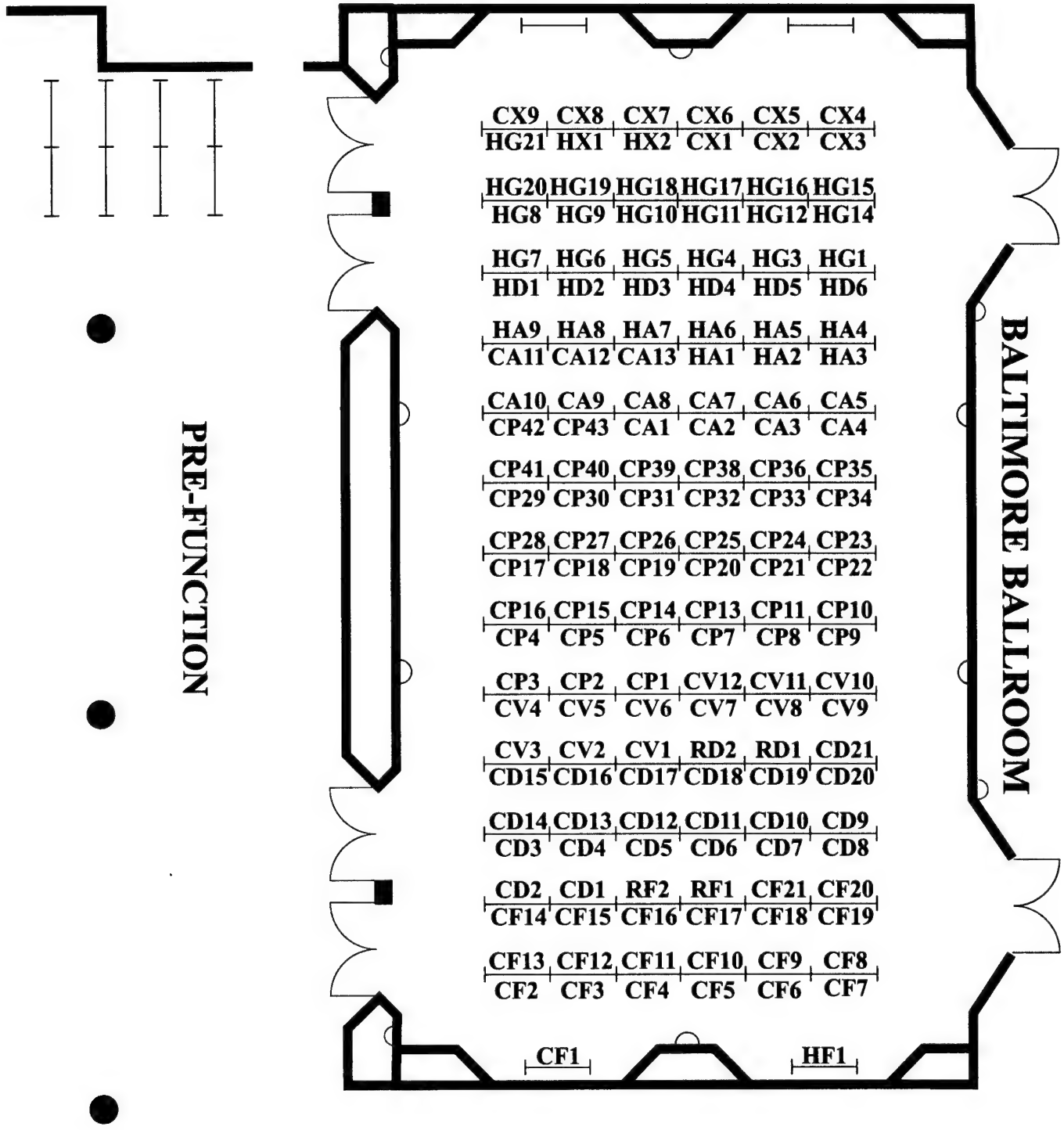
# Poster Session I

## Poster Placement (Monday/Tuesday)



# Poster Session II

## Poster Placement (Wednesday/Thursday)



# 10th International Workshop on Campylobacter, Helicobacter and Related Organisms

Renaissance Harborplace Hotel
Baltimore, Maryland
September 12-16, 1999

**Sunday, September 12, 1999**

12:00 pm – 7:00 pm Registration

4:00 pm – 7:00 pm Put up Posters for Session I

7:00 pm – 10:00 pm Opening Reception Renaissance Harborplace Hotel

## Monday, September 13, 1999

7:00 am – 5:00 pm Registration

8:15 am – 8:30 am Opening Remarks-Harry L.T. Mobley

8:30 am – 9:30 am Plenary Session 1
Identification of Virulence Determinants in Bacterial Pathogens
*John Mekalanos*

9:30 am – 10:00 am Break

10:00 am – 11:30 am Concurrent Sessions

Concurrent Session A-Pathogenesis/Genetics of Helicobacter I: Virulence Determinants
*Co-chairs: Trevor Trust and Sebastian Suerbaum*

- Structure, Expression and Biology of Helicobacter LPS
- Factors Influencing Acid Adaptation and Gastric Colonization of *H. pylori*
- Host Responses to Helicobacter Colonization
- Role of UreI in Urease Activity

Concurrent Session B- Diagnostics & Clinical (*Campylobacter*)
*Co-chairs: Bertil Kaijser and Albert Lastovica*

- Optimal Conditions for the Isolation of Campylobacter and Related Organisms
- PCR-based Methods for the Detection and Characterization of *Campylobacter* Spp.
- Phenotypic and Genotypic Characterization of *Campylobacter fetus* Subspecies

- Detection of Campylobacter in Foods
- Detection and Characterization of New *Campylobacter spp.*
- Clinical Significance of *Campylobacter concisus* Infection
- Detection and Prevalence of Ciprofloxacin-Resistant Campylobacter

Concurrent Session C- Food Safety of Campylobacter I
*Co-chairs: Norman Stern and Jan van der Plas*

- Epidemiology of *Campylobacter* among Broiler Operations
- Optimum Detection, including Colonizing Non-cultivables: Improved Media and Methods
- Risk and Protection Assessments

11:30 am – 1:30 pm — Poster Session Ia Presenters Stand with Their Posters
*Campylobacter Epidemiology/Subtyping, Helicobacter Immunology/Vaccines, and Helicobacter Epidemiology*

12:30 am – 1:30 pm — Lunch Foyer of Baltimore Ballroom

1:30 pm – 3:00 pm — Concurrent Sessions

Concurrent Session D- Guillain-Barré Syndrome I
*Co-chairs: Irv Nachamkin and Anthony Moran*

- Epidemiology of Ganglioside-mimicry in Campylobacter
- Evidence of Ganglioside Mimicry in *H. pylori* and Other Non-campylobacter Bacteria
- Methods for Detection of Ganglioside-mimicry in Campylobacter
- Evidence that Certain O Serotypes are Associated with Different Forms of GBS, Including Fisher Syndrome
- LPS Biosynthesis Genes Involved in Ganglioside-like Epitope Expression in Campylobacter

Concurrent Session E- Antimicrobial Agents: Mechanisms of Action and Resistance (Campylobacter and Helicobacter)
*Co-chairs: Diane Taylor and Jorgen Engberg*

- Susceptibility Testing of Campylobacter and Helicobacter
- Mechanisms of Antimicrobial Resistance in Campylobacter and Helicobacter
- Prevalence and Trends of Antimicrobial Resistance
- Non Antibiotic Antibacterials

Concurrent Session F- Epidemiology of Helicobacter I
*Co-chairs: Masahiro Asaka and Martin Blaser*

- Transmission of *H. pylori*
- Changing *H. pylori* Prevalence
- Genomic Stability and Variation for Typing Purposes
- Geographical Variation of Strains
- Markers of Virulence in *H. pylori*
- Other *Helicobacter* Species in Humans

| | |
|---|---|
| 3:00 pm – 3:30 pm | Break |
| 3:30 pm – 4:00 pm | Plenary Session 2<br>*Chair: Guillermo Ruiz-Palacios*<br><br>Historical Perspective of *Campylobacter*<br>*Jean Paul Butzler* |
| 4:00 pm – 5:00 pm | Interaction of Pathogenic Bacteria with Eucaryotic Cells<br>*Brett Finlay* |
| 5:00 pm – 5:30 pm | Presentation for Next Meeting Venue |
| 5:30 pm – 7:00 pm | Poster Session 1 Baltimore Ballroom |
| 7:30 pm –9:30 pm | Concert Reception and Dinner at the Peabody Conservatory Library |

## Tuesday, September 14, 1999

| | |
|---|---|
| 8:30 am –9:30 am | Plenary Session 3<br>*Chair: Pinaki Panigrahi*<br><br>Protein Secretion in Pathogenic Bacteria<br>*Steve Lory* |
| 9:30 am- 10:00 am | Break |
| 10:00 am – 11:30 am | Concurrent Sessions<br><br>Concurrent Session G- Population Genetics of Campylobacter and Helicobacter<br>Co-chairs: *Rick Meinersmann and Mark Achtman*<br>• Clonal, panmictic, epidemic and other Factors of Population Structure<br>• Population genetics of *H. pylori*<br>• Clonal structure of Campylobacter<br>• Assessing clonal structure<br>• Multiple sequence analysis and clonality<br><br>Concurrent Session H- Diagnostics and Clinical (Helicobacter)<br>*Co-chairs: Francis Megraud and Steve Czinn*<br>• Diagnosis of *H. pylori* in Children and the Elderly<br>• Antimicrobial Resistance and Clinical Outcome<br>• New Diagnostic Methods: PCR and Stool-based Diagnostic Tests |

Concurrent Session I- Food Safety of Campylobacter II
*Co-chairs: Diane Newell and Mogens Madsen*

- Role of Vertical Transmission in Poultry Infections
- Risk Factors for Poultry Infections and Recommendations to Make the Poultry Industry Eliminate or Reduce these Risks
- Food Sources which Contribute to Human Infections: Magnitude of the Problem and Variability Between Countries
- Viable-But-Non-Culturables: Assessment of Viability and Importance in Human and Animal Infections
- Measures to Take, from the Farm Gate to the Retail Shelf, to Eliminate or reduce Campylobacter Contamination of Food

11:30 am- 1:30 pm — Poster Session Ib Presenters Stand with Their Posters
*Helicobacter Pathogenesis/Animal Models, Campylobacter Genomics, and Guillain Barré Syndrome*

12:30 am –1:30 pm — Lunch Foyer of Baltimore Ballroom

1:30 pm – 3:00 pm — Concurrent Sessions

Concurrent Session J- Pathogenesis/Genetics of Campylobacter: Virulence Determinants and Animal Models
*Co-chairs: Carol Pickett and Mike Konkel*

- Adherence: How and What does *Campylobacter* see *in vivo*
- Campylobacter invasion: The Players
- Campylobacter invasion: The Mechanics
- The Epidemiology and Biology of Cytolethal Distending Toxin
- Biosynthesis and roles of LPS
- Advances in animal models of Campylobacteriosis

Concurrent Session K- Vaccine Development & Host Immune Response in Helicobacter
*Co-chairs: Agnes Labigne and John Nedrud*

- Mechanisms/Correlates of Protection after Immunization Versus Helicobacter Infections
- *H. pylori* Vaccine Antigens: Vaccine Antigen Characteristics, New Vaccine Antigen Candidates and Multiple Antigens
- Parenteral Versus Mucosal Routes of Immunization: Advantages, Disadvantages and Possible Use in Humans
- Adjuvants and Immune Response: Cholera Toxin, Heat Labile Toxin, and Alum and Effect on Humoral Versus Cellular Immunity
- Other Vaccine Delivery Strategies: DNA Vaccines, Microencapsulation, and *Salmonella* or Other Vectors
- *H. pylori* Immune and Inflammatory Responses

Concurrent Session L- Epidemiology of Campylobacter: Subtyping and Taxonomy
*Co-chairs: Stephen On and Peter Vandamme*

- Relationship of Strain Diversity Within the Host/Vector/Carrier/Food Supply to Campylobacter Epidemiology
- Evidence for Non-poultry Reservoirs of Human Campylobacter Infection
- Criteria to Identify Interstrain Relationship for Molecular Studies of Campylobacter
- New Typing Methods- the Holy Grail or the Emperor's New Clothes?
- Relevance of Taxonomic Studies to Epidemiologists

3:00 pm – 3:30 pm — Break

3:30 pm – 5:30 pm — "Campynet": a network for the standardization and harmonization of molecular typing methods for Campylobacters (Closed Meeting)
*Stephen On*

3:30 pm – 4:00 pm — Plenary Session 4
*Chair: Guerillimo Perez-Perez*

Historical Perspective of Helicobacter
*Adrian Lee*

4:00 pm – 5:00 pm — Autoimmune Disease & Molecular Mimicry
*Kai Wucherpfennig*

5:00 pm – 6:30 pm — Poster Session I

6:00 pm – 7:00 pm — Soccer Match at DuBurns Arena Sign Up Sheet at Registration

8:00 pm – 9:30 pm — Reception and Dinner at the National Aquarium in Baltimore

## Wednesday, September 15, 1999

8:30 am – 9:00 am — Plenary Session 5
*Chair: Paul Hoffman*

Genomics of Campylobacter & Helicobacter
*Brendan Wren*

9:00 am – 9:30 am — Population Genetics
*Jim Musser*

9:30 am – 10:00 am — Break

10:00 am – 11:30 am — Concurrent Sessions

Concurrent Session M- Vaccine Development & Host Immune Response in Campylobacter
*Co-chairs: Patricia Guerry & Charles Penn*

- Host Immune Response to Campylobacter
- Vaccine Development
- Human Challenge Studies with *Campylobacter jejuni*

Concurrent Session N- Diagnostics and Clinical II (Helicobacter)
*Co-chairs: Manfred Kist and Tim Cover*

- *Helicobacter* Infections of Humans with Species Other Than *H. pylori*: Gastric, Biliary, Intestinal and Other
- New Serological Diagnostics Tests for *Helicobacter pylori* infection
- Clinical Effects of *H pylori* Outside the Stomach and Duodenum: Esophageal Disease, Coronary Artery Disease and Growth Retardation
- Geographic Differences in Clinical Complications of *H pylori* Infection

Concurrent Session O- Genomics of *Campylobacter* and *Helicobacter*
*Co-chairs: Richard Alm and Julian Ketley*

- Comparison of the Campylobacter and Helicobacter Genome Sequences
- Genome Variation
- Transcript Profiling and Microarrays: Possibilities and Pitfalls
- Global Transcriptional Regulation: Regulators and Proteomics
- Iron Uptake and Regulation

11:30 am –1:30 pm — Poster Session IIa Presenters Stand with their Posters
*Food Safety, Campylobacter Pathogenesis/Animal Models, Helicobacter and Campylobacter Population Genetics, and Helicobacter Genomics*

12:30 am – 1:30 pm — Lunch Foyer of Baltimore Ballroom

1:30 pm –3:00 pm — Concurrent Sessions

Concurrent Session P- Non-gastric Helicobacters and Helicobacter Animal Models
*Co-chairs: Adrian Lee, Jim Fox and David Schauer*

- Modulation of polarized Th1 responses against *Helicobacter* species by parasites
- Isogenic mutant strains of *Helicobacter* species in normal or transgenic mice: Criteria to assess colonization and disease
- Enterohepatic Helicobacter species in human hepatitis, liver cancer, cholangitis, cholangiocarcinoma, IBD, and colon cancer
- Bacterial virulence determinants of enterohepatic *Helicobacter* species
- Increasing numbers of enterohepatic *Helicobacter* species

Concurrent Session Q- Guillain-Barré Syndrome II
*Co-chairs: Norman Gregson and Ben Appelmelk*

- Determinants Involved in the Development of GBS- Host Versus Bug
- Microbial Factors That Contribute to the Ability of Campylobacter to Cause GBS
- Host Factors That Contribute to Susceptibility for Developing GBS Following Campylobacter Infection
- Considerations for Establishment of an Animal Model of Campylobacter-Induced GBS

Concurrent Session R- Epidemiology of Helicobacter II: Typing
*Co-chairs: Robert Owen and Stuart Hazell*

- Choosing a Method for Strain Phenotyping of *H. pylori*
- *H. pylori* Genotyping: Utility of Markers Associated with the *cag* PAI
- *H. pylori* Genotyping: Pros and Cons of Genomic Macrodiversity Markers
- *H. pylori* Genotyping: Pros and Cons of Intragenic Diversity Markers
- Evidence for Population-Specific Strains of *H. pylori*
- Effect of *H. pylori* Genome Plasticity on Accuracy and Stability of Typing Systems
- Approaches for Typing Strains of Species of Helicobacter other than *H. pylori*
- Benefits of Consensus Guidelines for Typing *H. pylori* and Other Important Species

| | |
|---|---|
| 3:30 pm – 5:00 pm | Plenary Session 6<br>*Chair: Ban Allos* |
| 3:30 pm – 4:15 pm | Enteric Vaccines<br>*James Kaper* |
| 4:15 pm – 5:00 pm | Role of *Helicobacter pylori* in Development of Gastric Cancer<br>*Martin Blaser* |
| 5:00 pm – 6:30 pm | Poster Session II Baltimore Ballroom |
| 7:30 pm – 11:00 pm | Closing Banquet **Tickets Required** Renaissance Hotel |

## Thursday, September 16, 1999

8:30 am – 10:30 am

Poster Session IIb Presenters Stand with Their Posters
*Helicobacter and Campylobacter Antimicrobial Agents, Campylobacter Immunology/Vaccines, Helicobacter Diagnostics/Clinical, and Campylobacter Diagnostics/Clinical*

8:30 am – 10:30 am

Special Interest Working Groups (Open to All Interested)
ICBS Meeting
*Peter Vandamme*
Campylobacter Reference Lab Working Group
*Jennifer Frost*
Multi-locus Sequence Typing (MLST) of Campylobacter
*Rick Meinersmann*

10:30 am-12:00 pm

Selected Papers from Young Investigators
*Co-chairs: David McGee and Stuart Thompson*

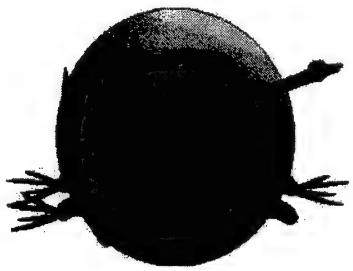
- Characterization of *pgl* mutants in an isolate of *Campylobacter jejuni* from a patient with Miller Fisher Syndrome
  *Christine Szymanski*
- *Campylobacter jejuni* lipopolysaccharides from Guillain-Barré Syndrome and Miller Fisher patients induce anti GM1 and anti-GQ1b antibodies in rabbits
  *Wim Ang*
- A new genotyping system for C. *jejuni* strains based on LPS genes
  *Benjamin Fry*
- Characterisation of the haemin uptake system of *Campylobacter jejuni*
  *Jonathan Rock*
- Characterization of the secretion of the Cia proteins from *Campylobacter jejuni*
  *Vanessa Rivera-Amill*
- Lectin typing of *Helicobacter pylori* from various geographical regions
  *Sean Hynes*
- Observations on regulation of gene expression in *Helicobacter pylori*
  *Beverly Davies*
- The use of *lacZ* and *cat* reporter genes in *Helicobacter pylori*: detection and quantification of the gene products with commercial ELISA kits
  *Stefan Bereswill*
- Gastric Homing of CD4+ cells in recepient SCID mice varies based upon infection status of the donor
  *Richard Peterson II*
- Dissecting the *Helicobacter pylori* vacuolating cytotoxin using sliding complementation analysis within target cells
  *Steven Blanke*

12:00 pm- 12:05 pm

Closing Remarks
Harry Mobley

# ✦Abstracts✦

| | |
|---|---|
| CA or HA | Antimicrobial Agents |
| CB | Guillain-Barré Syndrome |
| CD or RD | Campylobacter Diagnostics and Clinical |
| CE or RE | Campylobacter Epidemiology and Subtyping |
| CF, RF or HF | Food Safety |
| CG or RG | Campylobacter Genomics |
| CP | Campylobacter Pathogenesis and Animal Models |
| CV | Campylobacter Immunology and Vaccines |
| CX or HX | Population Genetics |
| HD | Helicobacter Diagnostics and Clinical |
| HE | Helicobacter Epidemiology |
| HG | Helicobacter Genomics |
| HP | Helicobacter Pathogenesis and Animal Models |
| HV | Helicobacter Immunology and Vaccines |



**CA1** Allicin: An Answer to Multiresistant Bacterial Infections.

**NOTES**

A. J. LASTOVICA,[1] D. SIDLER[2], P. DE WET[2], H. RODE[2]

[1]Dept. of Medical Microbiology, and [2]Dept of Paediatric Surgery, Institute of Child Health, Red Cross Children's Hospital, University of Cape Town, South Africa.

The ever increasing incidence of multi-antibiotic resistant bacterial infections world wide is of great concern. We suggest that serious consideration be given to the development of allicin, the active ingredient of *Allium sativum*, (garlic), as a supplementary form of treatment. The medicinal, and especially the antibacterial properties of garlic have been known since time immemorial. We have demonstrated the antibacterial properties of aqueous garlic extracts *in vitro*. The threshold concentration of garlic at which detectable inhibition occurred was 1/16 (0.4mg allicin/ml) for all Gram-negative organisms tested. Gram positive organisms were inhibited by a concentration of between 0.4 and 0.2 mg/allicin (1/16 and 1/32 dilution). Time course studies indicated that killing occurred within 10 hours for all Gram-positive organisms examined, and varied from 8 to 14 hours for Gram-negative organisms. No bacterial growth was observed after 14 hours exposure to the MBC of the garlic extract. Allicin has been effective in the *in vitro* eradication of a variety of *Campylobacter* spp. and *H. pylori*. The antibacterial effect of an aqueous garlic extract was investigated against 38 clinical isolates of *Campylobacter* from diarrhetic paediatric patients, and 32 isolates of *H. pylori* from adult patients with peptic ulcers. All isolates of *Campylobacter* and *H. pylori* tested were susceptible to allicin, and zones of inhibition ranged from 16 to 32 mm in diameter, with a mean of 21 mm. Subcultures from zones of inhibition produced no growth after 48 hours, suggesting that the mode of action of allicin against *Campylobacter* and *H. pylori* is bactericidal. These results suggest that allicin should be investigated further as a possible antibiotic treatment.

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**CA2** **Detection of a Repeat-Containing *dfr1* Cassette on The Chromosome Mediating Trimethoprim Resistance in *Campylobacter jejuni* .**

Amera Gibreel and Ola Sköld.

Division of Microbiology, Department of Pharmaceutical Biosciences, P.O. Box 581, Biomedical Center, Uppsala University, SE-751 23, Uppsala, Sweden.

The frequent occurrence of high level trimethoprim resistance in clinical isolates of *C. jejuni* was shown to be related to the acquisition of foreign resistance genes (*dfr1* or *dfr9* or both) coding for resistant variants of the enzyme dihydrofolate reductase, the target of trimethoprim. The occurrence of these trimethoprim resistance genes in *C. jejuni* could be a reflection of the massive use of trimethoprim both in human and veterinary medicine. In about 36% of the 41 examined isolates, the *dfr1* gene showed identity to the previously characterized gene in trimethoprim resistant *E. coli*. In 40% of the cases, however, a 90 bp repeat-containing variant of the *dfr1* gene was detected expressing a DHFR enzyme more susceptible to the drug than the type I DHFR enzyme characterized earlier. These two alleles of the *dfr1* gene was also found to exist in about 10% of the examined isolates. Moreover, in 5% of the isolates, the repeat-containing *dfr1* variant was found to occur in the form of two cassettes in tandem located in an integron context. Under the selection pressure of trimethoprim, the 90 bp repeat within the coding sequence of the *dfr1* gene seemed to mediate the generation of other DHFR size variants in the population. One of the resulting variants, showing the deletion of the 90 bp repeat from the coding sequence of the gene, was found to mediate the resistance to a high concentration of trimethoprim and hence conferring a selective advantage. These results suggest that the repeat-containing *dfr1* gene might play a role in the adaptability of *C. jejuni* isolates to the variation in the environmental selection pressure. The contribution of Tn*7* and Tn*21* to the high incidence of the *dfr1* gene on the *C. jejuni* chromosome was investigated using a PCR approach. The most common combination was *dfr1* and *int*-Tn*21* indicating a high prevalence of Tn*21*.

**NOTES**

Antimicrobial resistance of 219 human strains of *Campylobacter jejuni* subsp. *jejuni* isolated from 1995 to 1998. CA3

C. GAUDREAU*, H. GILBERT, Département de Microbiologie médicale et infectiologie, Hôpital St-Luc du CHUM, Montréal, Québec, Canada.

The aim of the study was to determine the resistance of 219 strains of *C. jejuni* to erythromycin (E), tetracycline (T) and ciprofloxacin (C) using an agar dilution method and the resistance to nalidixic acid (NA) using a disk diffusion method. The rate of resistance of *C. jejuni* strains to four antibiotics were:

| | 1995 (n=52)* %R** | 1996 (n=51) %R | 1997 (n=55) %R | 1998 (n=61) %R | 1995-1998 (n=219) %R |
|---|---|---|---|---|---|
| Erythro | 0% | 0% | 0% | 3.3% | 0.9% |
| Tetra | 57.7% | 66.7% | 45.5% | 42.6% | 52.5% |
| Nalid. acid | 7.7% | 21.6% | 12.7% | 9.8% | 12.8% |
| Cipro | 5.8% | 19.6% | 12.7% | 9.8% | 11.9% |

*: one strain from each patient was tested; ** % of resistance

There was a significant reduction of the resistance to T in 1997 and 1998 in comparison to the one obtained in 1995 and 1996, P=0.026. There was no significant difference in the annual resistance or in the resistance trend for E, C and NA. The $MIC_{50}$ and $MIC_{90}$ were 0.5 and 2 $\mu$g/ml for E, 16 and >128 $\mu$g/ml for T, ≤0.06 and 4 $\mu$g/ml for C. One hundred and ninety one strains with a zone to NA disk were susceptible to C using the agar dilution. Of the 28 strains without a zone to NA, 26 and 2 were respectively resistant and intermediate to C. Thirteen of 26 *C. jejuni* resistant to C and 103 of 191 *C. jejuni* susceptible to C were resistant to T (P=0.7). From 1995 to 1998, for 219 human *C. jejuni*, there were stable levels of resistance to erythromycin, nalidixic acid and ciprofloxacin but a significant reduction in the levels of resistance to tetracycline. Nalidixic acid disk was a good marker for susceptibility to ciprofloxacin. There was no association between ciprofloxacin and tetracyclin resistance.

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**Increased Incidence of Quinolone Resistance Among Clinical Isolates of *Campylobacter jejuni* in Mexico.** CA4

**TUZ F**[1]., GUERRERO M.L.[1], CERVANTES LE.[1], PICKERING L.K.[2], RUIZ-PALACIOS G.M.[1]

National Institute of Nutrition, Mexico City, Mexico[1]. Center for Pediatric Research, Norfolk, USA[2]

Increasing incidence of quinolone resistance among Campylobacter clinical isolates from humans and animals has been observed in Europe, and recently in the United States among travelers to Mexico. It is suggested that veterinary use of quinolones induces selective pressure for the emergence of resistance to ciprofloxacin (CIPRO) among human isolates. Quinolone-resistant Campylobacter strains have not been reported in Mexico. In this study, we evaluated resistance to quinolones among Campylobacter clinical isolates from Mexico obtained from 1986 to 1997.

381 *C.jejuni* strains isolated during 1986-1997 from children were tested for resistance to nalidixic acid (NA), CIPRO and erythromycin (ER) by the E test and broth dilution MICs. A good correlation was observed between both tests. 57% of strains were resistant to CIPRO and 62% to NA, with MICs =>256 ug/mL. Crossed resistance between NA and CIPRO was found in 92% of isolates. Only 2.4% of strains were resistant to ER. The increase in NA- and CIPRO-resistant *C.jejuni* found in this study suggests that both antibiotics may have limited use for the treatment of *C.jejuni* intestinal infections in Mexico.

**CA5** Antimicrobial resistance among human *Campylobacter* isolates in the United States, 1997 - 1998

**NOTES**

MARANO N[1], STAMEY K[1], BARRETT TJ[1], ANGULO FJ[1], and the NARMS:Enteric Bacteria Working Group
[1]Centers for Disease Control and Prevention, ATLANTA, Ga.

Antimicrobial agents, while not essential for most persons with *Campylobacter* enteritis, shorten the illness duration and reduce the severity of symptoms if given early in the course of the infection. Erythromycin and fluoroquinolones (e.g.ciprofloxacin) are most commonly used to treat persons with campylobacteriosis. As campylobacteriosis is usually a zoonotic infection, antimicrobial resistance among human *Campylobacter* isolates is likely to be due to the use of these antimicrobial agents in food-producing animals. To monitor the prevalence of resistance among human *Campylobacter* isolates, seven participating state public health laboratories forward the first *Campylobacter* isolate received each week to the Centers for Disease Control and Prevention (CDC) as part of the National Antimicrobial Resistance Monitoring System (NARMS):Enteric Bacteria surveillance system. At CDC, these isolates are tested for antimicrobial susceptibility to chloramphenicol, ciprofloxacin, clindamycin, erythromycin, nalidixic acid, and tetracycline. Identification of *Campylobacter* is performed using dark-field motility, oxidase test, and hippurate test. In 1997-1998, 563 *Campylobacter* isolates were tested; 535(95%) were *C. jejuni*. Among *C. jejuni* isolates, 250 (47%) were resistant to tetracycline, 97 (18%) to nalidixic acid, 71(13%) to ciprofloxacin, 24 (5%) to erythromycin, 14 (3%) to clindamycin, and 13 (2%) to chloramphenicol. Three *C. jejuni* isolates were resistant to both ciprofloxacin and erythromycin. We conclude that a high proportion of *Campylobacter jejuni* isolates are resistant to antimicrobial agents commonly used for treatment of campylobacteriosis. The level of resistance to these agents indicates the need for safeguards to be established, and that all stakeholders (public health officials, veterinarians, and the food animal and pharmaceutical industry) work together to preserve the efficacy of these agents for when their use is critical.

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**CA6** Erythromycin Resistance in Campylobacter

C. A. TRIEBER and D. E. TAYLOR
University of Alberta, Edmonton, AB Canada

*Campylobacter* spp., primarily *C. jejuni* and less frequently *C. coli*, are a major cause of human bacterial diarrhea. The drugs of choice for treatment of these intestinal pathogens are the macrolides, such as erythromycin and clarithromycin. With usage of these antimicrobial agents has come the emergence of erythromycin resistant clinical isolates. Common mechanisms of resistance such as efflux or methylation of the cellular target of erythromycin, the 23S rRNA, has not been found in either *C. jejuni* or *C. coli*. We have sequenced the 23S rRNA genes from several clinical isolates and found that the majority of erythromycin resistant isolates contain nucleotide changes at residues in Domain V of the 23S rRNA genes when compared with erythromycin sensitive isolates. These residues are equivalent to A2057 and A2058 in the *E. coli* 23S rRNA which are involved in the binding of erythromycin to the ribosome. The erythromycin resistance phenotype is transferable by natural transformation of sensitive *Campylobacter* spp. strains with chromosomal DNA from resistant organisms. A range of erythromycin resistance levels are displayed, and correspond to the particular mutation present. This mechanism of macrolide resistance is identical to that found in *Helicobacter pylori*.

**NOTES**

CA7

Campylobacter Enteritis: Laboratory Aspects Concerning Primarly Antimicrobial Agents

CROTTI D., FONZO G., D'ANNIBALE M.L., MEDORI M.C., LUZZI I.°
Hospital of Perugia, °Health Institute of Roma, Italy

**Objectives:** to survey antibiotic resistances and to validate direct erythromycin sensitivity "in vitro".

**Methods:** during 96-98 1375 stool specimens were processed. Liquid faeces were processed using double filter membranes, with and without erythromycin disc on blood/charcoal agar. Not formed faeces were processed using conventional methods. Antimicrobial sensitivity was performed/confirmed by a modified agar diffusion test on blood agar.

**Results:** 133 strains (9.6%) of Campylobacter were isolated: 83 C.jejuni and 50 C.coli. Direct and confirmed erythromycin resistances were similar: 9.8%(16% in C.coli, 6% in C.jejuni). Other resistances were: to norfloxacin 42.9%(46 and 41 %), to tetracycline 47.4%(44 and 49.4%), to minocycline 25.6 %(26 and 25.3%), to amoxicillin plus clavulanate 9%(12 and 7.2%). No resistances were observed to gentamicin and chloramphenicol.

**Conclusions:** quinolones resistances are increased again. Resistances to erythromycin are low. Direct erythromycin sensitivity test is useful.

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CA8

*Trichuris suis* Excretory-Secretory Products Inhibit the Growth of *Campylobacter jejuni*. S. R. ABNER[1], D. E. HILL[2], G. PARTHASARATHY, and L. S. MANSFIELD[1] Michigan State University, East Lansing, MI[1] and USDA, Beltsville, MD[2].

We have developed a swine animal model in which natural host resistance to *Campylobacter jejuni* is altered by experimental infection with low numbers of the nematode *Trichuris suis*, whipworm. Pigs colonized with *C. jejuni* experience colitis due to the invasion of the bacterium approximately 21 days following exposure to *T. suis*. While performing experiments to test the hypothesis that *T. suis* excretory-secretory products (ESP) render intestinal epithelial cells more permissive for *C. jejuni* invasion, an antibacterial activity was identified in the ESP. Precedent for antibacterial activity exists in another nematode, *Ascaris suum*. Antimicrobial susceptibility disks were saturated with ESP at various concentrations and applied to plates inoculated confluently with *C. jejuni* for a standard disk diffusion assay. There was a dose-dependent inhibition of growth proportional to the concentration of ESP applied. Although it was considered unlikely that residual antibiotics from *in vitro* culture of whipworms could account for *C. jejuni* growth inhibition, liquid chromatography-mass spectrometry (LC-MS) was performed to exclude that possibility. As expected, none of the antibiotics used were detected in the LC-MS assay. Using a standard broth microdilution method, the minimum inhibitory concentration of ESP was determined to be 25 μg/ml for multiple *C. jejuni* isolates, whereas *C. coli* was higher at 100 μg/ml. This activity is heat stable, remains after freezing-thawing, and is resistant to alkaline and acid pH. Reverse phase high performance liquid chromatography (HPLC) was performed using a C18 column to fractionate ESP peptides. The antibacterial activity has been localized to two hydrophilic peptides. Additional HPLC control experiments are in progress.

CA9

## Intracellular Redox State and Disulfide Reduction in *Campylobacter* Spp.

NOTES

G. L. MENDZ, M. A. SMITH, C. M. GRANT
*The University of New South Wales, Sydney, AUSTRALIA*

Intracellular redox homeostasis is regulated in most organisms by low molecular weight thiol compounds, which can take part also in the response to oxidative stress by eliminating harmful oxidants. Most species of the genus *Campylobacter* require a microaerobic environment for growth; and the metabolism of oxygen, even at low tensions, is a source of cytotoxic reactive oxygen species. To understand the microaerophily of these bacteria, the mechanisms they employ to regenerate the reducing conditions in the cytosol and defend themselves against toxic oxygen products were investigated.

Study of redox-active intracellular compounds by electrochemical HPLC detection showed the presence of significant concentrations of L-cysteine and several other endogenous metabolites. Although the bacteria are capable of taking up and metabolizing exogenous glutathione, its intracellular levels in cells grown in glutathione-free media were negligible. Very active thiol oxidoreductases catalyzing the reduction of thiol disulfides, in particular L-cystine, with NADH or NADPH as proton donors were measured employing nuclear magnetic resonance spectroscopy. Some of these enzyme activities were localized in the cytosolic fraction and others in both the cytosolic and cell-envelope fractions obtained by centrifugation of whole cell lysates.

The results suggested that L-cysteine plays a role similar to glutathione in other prokaryotes and eukaryotes in the maintenance of redox state and protection against oxidative challenge.

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CA10

## In vitro study the effect of organic acids on *Campylobacter spp.* population in the mixture of water with feed.

**P. Chaveerach, D.A. Keuzenkamp, H.A.P. Urlings and L.J.A. Lipman.**
Department of Science of Food of Animal Origin, Faculty of Veterinary Medicine, Utrecht University, The Netherlands.

Gastroenteritis caused by campylobacter infection has been recognized as one of the important public health problems in the developed countries. Outbreaks mostly originate from the consumption of contaminated poultry. Biosecurity strategies on farm level should be reconsidered seriously to reduce the amount of contamination.

The aim of this current study is to determine the bactericidal activity on campylobacter of organic acids individually and in combinations at different pH levels and times. Ten strains of campylobacters were added in a mixture of water with commercial broiler feed separately adjusted by four acids: formic, acetic, propionic and hydrochloric acids, into pH 4.0, 4.5, 5.0 and 5.5. The combination of three organic acids was used in two different formulas; formic: acetic: propionic as 1:2:3 and 1:2:5, at pH 4.0, 4.5, 5.0 and 5.5. All organic acids at pH 4.0 showed strong bactericidal effect on campylobacter, at pH 4.5. The reduction rate in 1h was 2.5 log, 2.4 log and 2.0 log respectively ($P<0.05$). In contrast at pH 5.0 and pH 5.5 the bactericidal activity of the four acids was low. The combination of formulas showed a synergic bactericidal activity increased the reduction rate in 1 h as 3.2 log and 3.0 log ($P<0.05$) at pH 4.5.

Therefore it can be concluded that organic acids individually or in combination have a bactericidal effect on campylobacter. Routinely appliance of organic acids in water supply on poultry farms could prevent campylobacter colonization.

CA11

## The in vitro activity of newer fluoroquinolones against *Campylobacter jejuni/C. coli* and *Helicobacter pylori*

R. KRAUSSE, S. SCHUBERT, U. ULLMANN, Dept. of Medical Microbiology & Virology, University of Kiel, Germany

The new fluoroquinolones exhibit good activity against gram-negative and some -positive bacteria, however their activity against *Campylobacter* and *Helicobacter* is not well studied. There is a great need for new antibiotics to treat infections caused by these bacteria that do not respond to the commonly used drugs.
The objective of this study was to investigate the susceptibility of *Campylobacter jejuni/C. coli* as well as *Helicobacter pylori* to Ciprofloxacin (CIP), Gatifloxacin (GTX), Grepafloxacin (GPX), Levofloxacin (LFX), Moxifloxacin (Bay 12-8039) (MFX) and Trovafloxacin (TFX).
The minimal inhibitory concentrations (MICs) (range 0.001-128 mg/l) of *C. jejuni/C. coli* (n= 35) and *H. pylori* (n= 13), non-copy isolates from faeces and biopsy material (antrum/corpus) of symptomatic patients, were determined using the agar plate dilution method on Brain-Heart-Infusion (BHI)-agar supplemented with 7 % sheep blood.
**Results:** $MIC_{90}$ values (mg/l)

| bacteria | CIP | GTX | GPX | LFX | MFX | TFX |
|---|---|---|---|---|---|---|
| *C. jejuni/C.coli* | 0.5 | 0.25 | 0.5 | 1.0 | 0.25 | 0.125 |
| *H. pylori* | 0.5 | 2.0 | 4.0 | 4.0 | 8.0 | 4.0 |

**Conclusions**
With the exception of levofloxacin all the other fluorochinolones showed good activity against *C. jejuni/C. coli*. With regard to *H. pylori*, ciprofloxacin was the only considerable agent. The newer fluorochinolones could be used to treat infections with *C. jejuni/C. coli* but not infections with *H. pylori*.

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CA12

## Acquisition of Quinolone Resistance and Point Mutation of *gyr*A Gene in *Campylobacter jejuni* Isolated from Broilers and *in vitro*-induced Resistant Strains

T. CHUMA, H. NIWA, T. MAEDA, and K. OKAMOTO
Dept. of Veterinary Public Health, Kagoshima University, Kagoshima, Japan

There has been a drastic rise in the resistance of *Campylobacter* to quinolones in human patients in Japan as well as in some other countries. In Japan, quinolones are not used as feed additives in broiler farms but are commonly used for the treatment of infectious bacterial diseases in broilers. In this study, the prevalence of quinolone resistant *C. jejuni* and *C. coli* in broilers was investigated and the mechamism of the acquisition of quinolone resistance was studied by detecting the point mutation in *gyr*A gene of the strains obtained from broilers and in vitro-induced resistant strains.
*C. jejuni or C. coli* was isolated from 39 of 150 cecal contents of broilers (12 of 30 farms). Among these strains, 7 strains (3 farms) were nalidixic acid resistant.
MICs of nalidixic acid, ofloxacin, and norfloxacin against quinolone resistant strains induced from *C. jejuni* ATCC 33560 differed depending on the location of the point mutation of *gyr*A gene. All quinolone resistant strains obtained from broilers also had a point mutation.
These results suggest that quinolone resistant *C. jejuni* has spread in broilers in Japan and the level of the quinolone resistance may be involved with the location of the point mutation of *gyr*A gene.

CA13

## Comparison Antimicrobial Susceptibility Testing and Molecular Analysis of Clinical Isolates of Thermophilic *Campylobacter* Spp in Australia.

D. ALFREDSON[1]*, and V. KOROLIK[2]. Gold Coast Hospital, Southport, Qld., Australia[1], and Griffith University, Gold Coast Campus, Southport, Qld., Australia[2].

Thermophilic *Campylobacter* spp., such as *C.jejuni* and *C.coli*, are recognised worldwide as major causes of acute gastroenteritis in man. As with other bacterial causes of diarrhoea, susceptibility testing of campylobacters is often not performed in the clinical laboratory. The aim of this study was to evaluate disc diffusion and E-test in comparison with the agar dilution method for antimicrobial susceptibility testing of human clinical isolates of *C. jejuni* and *C. coli* with particular reference to ampicillin, erythromycin, and tetracycline. Further, PCR and restriction endonuclease (RE) fragment profiles were used to speciate and to differentiate individual strains. The Minimum Inhibitory Concentration (MIC) of 181 clinical isolates of *C. jejuni* and *C. coli* were determined by agar dilution and compared to disc diffusion susceptibility and E-test. Agar dilution however showed only 22% correlation with E-test for ampicillin; 100% for erythromycin; and 64% for tetracycline. The disc diffusion method correlated with agar dilution. PCR analysis determined that 78 (86%) of isolates were *C. jejuni* and 13 (14%) were *C. coli*. RE profiles showed 34 (37%) of the isolates formed 10 separate clusters of multiple isolations. Overall, the results suggest that disc diffusion is a reliable method of susceptibility testing for thermophilic *Campylobacter* spp. for erythromycin and tetracycline. E test was not reliable for determination of ampicillin and tetracycline resistance. PCR test and RE profiles enabled accurate speciation and differentiation of individual strains.

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HA1

## Characterisation of the *rdxA* gene and the gene encoding the NAD(P)H-flavin oxidoreductase (*flav*) in metronidazole resistant *Helicobacter pylori* strains.

C. BILARDI[1], A. MARAIS[1], C. BIRAC[1], V. SAVARINO[2], F. MÉGRAUD[1], Laboratoire de Bactériologie, Université *Victor Ségalen* Bordeaux 2, Bordeaux, France[1], Unità Operativa di Gastroenterologia, Università di Genova, Genova, Italia[2].

Metronidazole resistance is a major cause of failure of *Helicobacter pylori* eradication therapy. Several authors have associated this resistance with point mutations located in the *rdxA* gene. Other enzymes, belonging to the nitroreductase family, may be involved in the resistance to this antibiotic. To evaluate their role in resistance, both the *rdxA* and *flav* genes were amplified and sequenced in 9 paired *H. pylori* strains isolated before and after treatment including metronidazole. Each pair was verified to be identical by RAPD and their metronidazole sensitivity was determined by agar dilution method. The results are shown in the following table:

| Strains | Mutation | |
|---|---|---|
| | Flav | RdxA |
| 1 | No Mutation | Frame shift |
| 2 | 97 A → E | 108 Y → Stop |
| 3 | 62 D → Y | No Mutation |
| 4 | 40 A → V | 31 T → E |
| 5 | N.D. | 16 R → C |
| 6 | N.D. | No Mutation |
| 7 | N.D. | 57 S → L |
| 8 | N.D. | 84 Y → Stop |
| 9 | N.D. | 99 L → Stop |

N.D. = Not determined

These results confirm that the *rdxA* gene is involved in metronidazole resistance. However, in certain cases, point mutations located in the *flav* gene can equally be implicated in this resistance. Further experiments are undertaken to prove its role in this resistance.

HA2

## Antibiotic Resistance of Attached and Free-floating *H. pylori*

**Joanne L. Simala-Grant*, David Zopf+, and Diane E. Taylor***
***University of Alberta, Edmonton, Alberta, Canada**
**+ Neose Technologies, Inc., Horsham, Pennsylvania, USA.**

*Helicobacter pylori* colonizing the human stomach attaches to mucous cells or occurs in motile phase within the gastric mucous layer. There is abundant evidence that bacteria growing in biofilms, or attached to host eukaryotic cells show morphological and biochemical differences from their free-floating counterparts. Laboratory models able to mimic the *in vivo* metabolic state of microbes, are valuable to the extent that they create controlled conditions in which to study alterations in antibiotic resistance that may occur *in vivo*. Models that mimic attached and free-floating states may provide an accurate estimate of differences in MIC due to bacterial attachment, and are better suited for MIC measurements of *H. pylori*. For example, Megraud et. al compared amoxicillin resistance of free-floating *H. pylori* and those attached to HEp-2 cells (Antimicrob. Ag. Chemother. 35:869,1991). They demonstrated increased amoxicillin resistance of attached *H. pylori*.

We have modified Megraud's model so that free-floating and attached *H. pylori* are treated in a more similar manner, prior to, and after incubation with antibiotic. Killing curves were performed using 0, 0.001, 0.01, 0.1, and 10 μg/mL amoxicillin. We found both adherent and free-floating *H. pylori* remained viable, and in fact divided, at concentrations less than or equal to 0.01 μg/mL. In contrast to the results of Megraud et al, we observed no difference in resistance to amoxicillin between HEp-2 adherent versus free-floating *H. pylori* (26695). We find no evidence that attachment of *H. pylori* to eukaryotic cells *in vitro* increases their resistance to antibiotics compared to non-attached bacteria.

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HA3

## A *Helicobacter pylori* quinone- and nitro-reductase

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Quinone reductases are able to reduce quinone drugs by either a one- or a two-electron reduction process. One electron reduction produces a semi-quinone radical which can lead to the formation of oxygen free radicals. Two electron reduction produces the quinol form and consequently acts as a mechanism of detoxification.

Several cytosolic proteins with NAD(P)H:menadione reductase activity were detected in the bacterium *Helicobacter pylori* using a quinone reductase staining technique for native-PAGE. The most active quinone reductase was present in all the strains tested. This enzyme was purified employing ion-exchange and affinity chromatography. N-terminal sequence analysis of the purified enzyme gave an exact match to the predicted products of genes *hp0630* and *jhp573* of *H. pylori* strains 26695 and J99, respectively. The enzyme is homologous to the modulator of drug activity (MdaB) protein encoded by the gene *mdaB* in *E. coli* K-12, which is an NADPH-specific quinone reductase. Characterization of the purified *H. pylori* MdaB indicated it is a NADPH:quinone reductase, reducing menadione with a higher specificity for the electron donor NADPH than for NADH. The enzyme is able to use a wide range of electron acceptors including nitrofurazone and oxygen, indicating that it has also nitroreductase and oxidase activities. The primary function of the *H. pylori* MdaB appears to be quinone reduction, since this activity is approximately 10-fold and 50-fold higher than the oxidase and nitroreductase activities, respectively.

The ability of the enzyme to reduce quinone and nitro compounds may be significant in the detoxification/activation of quinone drugs and the activation of nitro compounds such as nitroimidazoles.

HA4 **Effect of Metronidazole on the Respiratory Chain of *Helicobacter Pylori***

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Metronidazole is one of the most commonly used drugs for the treatment of *Helicobacter pylori* infection. Understanding the mechanism of action of metronidazole may help to control the infection and the resistance problem. The effect of metronidazole on the respiratory chain of *H. pylori* has been investigated. Twelve strains of *H. pylori* have been used in this study, five of which are metronidazole-resistant. Metronidazole exhibited a concentration-dependent inhibitory effect on the activity of NADH-fumarate reductase of *H. pylori*. The $IC_{50}$ values of metreonidazole for fumarate reductase of the tested strains were in the range of 10 to 70 μM. There were no relationship between the $IC_{50}$ values of metreonidazole for fumarate reductase and metronidazole-resistance pattern. Metronidazole had no effect on the succinate dehydrogenase, NADH dehydrogenase, succinate-cytochrome *c* reductase and NADH-cytochrome *c* reductase of *H. pylori*. The data clearly demonstrate that metronidazole selectively inhibits the fumarate reductase of *H. pylori*.

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HA5 AP-PCR analysis of Helicobacter pylori strains from unsuccessful cases and recrudescence cases in eradication therapy

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Triple therapy using PPI, amoxicillin and clarithromycin is presently the best regiment for the treatment of Helicobacter pylori infection. However, we recognized some unsuccessful or recrudescence cases in eradication therapy. We attempted to analysis using AP-PCR method whether H. pylori infection were occurred with a single or multiple strains.

AP-PCR fingerprints of 20 isolates of H. pylori obtained from each of 10 patients showed same DNA patterns, respectively. The isolates from pre-eradication and post-eradication therapy of 13 unsuccessful cases in which the sensitivity of clarithrimycin had changed during eradication showed also identical patterns by AP-PCR analysis in each patients, respectively. On the other hand, the different pattern among a first and second isolates were observed in two recrudescence cases. Those results showed reinfection were occurred in those cases.

Conclusion: The present results suggest that almost patients may be exposed and colonized by one unique strain. But some patients may be infected with multiple strains or reinfected with other type of organisms.

## Effects of Astaxanthin and Vitamin C Against *H. pylori* Infection in Balb/cA Mice

HA6

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**Background:** *Helicobacter pylori* infections in human is associated with chronic type B gastritis, peptic ulcer disease and gastric carcinoma. A high intake of carotenoids and vitamin C have been proposed to prevent development of gastric malignancies. **Aim:** To explore if the micro algae *Haematococcus pluvialis* rich in the carotenoid astaxanthin and vitamin C can inhibit experimental *H. pylori* infections in a BALB/cA mouse model. **Materials and Methods:** Six-week-old BALB/cA mice were infected with a mouse passaged *H. pylori* strain 119/95. At two weeks post-inoculation mice were treated orally once daily for 10 days with different doses of astaxanthin (10, 50 and 100 mg/kg body weight) or vitamin C (400 mg/kg body weight). Half of the animals from each group were sacrificed immediately after the treatment and the other half were sacrificed 10 days later (n=10 per group). Gastric culture and histopathology were used to follow this study. **Results:** Mice treated with astaxanthin or vitamin C showed significantly lower colonization compared to the *H. pylori* infected control animals immediately after treatment and 10 days post-treatment. The inflammation score was significantly lower in the astaxanthin or vitamin C treated animals than in the infected control mice. A dose of 100 mg/kg of astaxanthin was found to be the most effective compared to 10 and 50 mg/kg body weight. However, an inhibitory effect was even seen with the lowest dose (10 mg/kg). **Conclusion:** Nature antioxidants may be a new and exciting field for treating *H. pylori* induced infections in humans.

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## Stability of Clarithromycin Resistance in *H. pylori* Strains and Effect of Sub-inhibitory Concentrations of Macrolides

HA7

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The influence of the MIC level and the type of mutation on the 23S *rRNA*, in the loss of *in vitro* clarithromycin resistance after several subcultures in the laboratory were determined. The effect of macrolides subinhibitory concentrations in the development of resistance was also studied. Thirty clarithromycin resistant *H. pylori* strains were selected by agar dilution and subcultured every 2 to 3 days for 10 to 20 times in a free-drug media. MIC was determined each 5 times to detect *in vitro* stability. Mutations in the *rRNA* gene were detected by a PCR-RFLP (*BsaI* and *MboII* to detect A2143G and A2142G, respectively). Clarithromycin MIC ranged from 8 to 64 mg/L. Three out of the 30 (10%) clarithromycin resistant strains tested lost the resistance after 10, 13 or 18 subcultures (MIC changed from 8 to 0.008, from 16 to 0.064 and from 32 to 0.016mg/L). Resistance was unstable in 1/17 strains with MIC 8mg/L, 1/6 with MIC 16mg/L and 1/4 with MIC 32mg/L. Resistance was unstable in 2/7 strains with A2142G and in 1/20 with A2143G mutations.
*H. pylori* strain NCTC 11638 and TIGR 26695 were cultured for several times on media containing macrolides and resistance was determined by MIC and PCR-RFLP. No significant changes on the macrolide MIC values were observed either at 1/2 or 1/8 MIC concentration up to 10 days. A change on the MIC was observed only when NCTC 11638 was exposed to 1/2MIC of erythromycin for 20 days. A genomic study of the colonies showed an A2143G mutation in the strain.
Conclusions: It is important to know the *in vitro* effect of the subinhibitory concentrations of macrolides as well as the stability of this resistance in order to study the repercussion in the clinical practice.

HA8

## Determination of Clarithromycin Resistance in *Helicobacter pylori* Clinical Isolates by Disc Diffusion. E-test and Agar Dilution.

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Background: An agar dilution method is recommended for *H. pylori* susceptibility testing, however, some times disc diffusion or E-test are used. Although many studies of discrepancies with metronidazole are available no many are for clarithromycin.
The aim of this study was to determine the *in vitro H. pylori* susceptibility comparing agar dilution with disk diffusion or E-test for clarithromycin (CLA).
Methods: 183 *H. pylori* clinical isolates were obtained from gastric biopsies taken at routine endoscopy. An agar dilution method using Mueller Hinton agar supplemented with horse blood was performed as the reference method. Resistance to CLA was considered when MIC $\geq$ 4 mg/L. A disk diffusion method was carried out in blood agar plates with 15mcg CLA in 105 strains and resistance considered when inhibition zone was <30mm for clarithromycin. An E-test method was performed according to manufacturer recommendations in 78 strains. A high number of CLA resistant strains were included in both groups.
Results: When disc diffusion was compared with the reference method in 105 strains, resistance to CLA was found in 24.7% by agar dilution and 20% by disc diffusion. In 89.6% a good agreement was found with A 2.8% of major and 7.6% of very major error. When E-test was compared with agar dilution in the 78 strains, resistance was detected in 32% by agar dilution and 29.5%, In 94.7% a good agreement was found with 1.3% of major and 4% of very major error.
Conclusions: In this study we demonstrate that E-test showed less error than disc diffusion to determine *in vitro* susceptibility of clarithromycin. Agar dilution, although more laborious and time-consuming method should be used in order to avoid misidentification of susceptibility/resistance.

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HA9

## Study Of The Tolerance To Amoxycillin In *Helicobacter pylori*

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The aim of this study was to investigate *in vitro* the phenomenon of the tolerance (T) to amoxycillin (A) in clinical isolates of Helicobacter pylori ( Hp).We selected 27 strains of Hp.The isolates were obtained by endoscopy from patients suffering different peptic diseases and stored at -80ºC during three years.
The MIC was determined by agar dilution as recommended. To study the tolerance the MBC was performed by a microdilution broth method. Several two-fold dilutions of A (from 128 mg/L to 0,008 mg/L) were prepared with BHI broth containing 10% of foetal calf serum. The T was defined with the final inoculum of $10^5$ ufc-$10^6$ ufc/ well. The microplates were incubated at 37ºC in a microaerobic atmosphere. After 72 hours 10 µl of each well was cultured on blood agar A free plates and incubated 72 h. The T was classically defined by MBC/MIC $\geq$ 32, but $\geq$8 could also be considered.In 5 Hp A sensible we performed the transformation with DNA from one strain tolerant.
**Results:** •There were 7 strains tolerant to A (25.9% ):6 with CMB/CMI $\geq$ 32 and 1 with CMB/CMI=16 .The 71,4% the strains tolerant had CMI $\geq$ 0,1 mg/l and the 28,5% had CMI $\leq$ 0,008 mg/L. In others 4 strains (14,8%) with CMI $\geq$ 0,1 mg/L. we observed a "persistence" phenomenon when the initial inoculum was $\geq$ $7x10^7$ cfu/ml, a small proportion of cell survives to antibiotic concentration $\geq$ 16 mg/L (less than 0,1% of inoculum).
•In the transformation assay any of 5 strain recipients uptook the DNA.
**Conclusions**: We found persistence and tolerance phenomenon more frequently in the strains with CMI $\geq$ 0,1 mg/l. The true incidence and clinical implications of tolerance among *H. pylori* isolates remains to be determined, to performance CMB seems to be the better tool *in vitro* for continuos surveillance of susceptibility of *H. pylori* to A.

NOTES

CB1

## Prevalence of the Gm1 Ganglioside Epitope in South African Clinical and Animal Isolates of *Campylobacter, Heliocobacter & Arcobacter*

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Some *Campylobacter* strains associated with the Guillain-Barré syndrome (GBS) have the Gm1 epitope in their lipopolysaccharide. Infection with these strains may induce GBS by an autoimmune process (molecular mimicry). The distribution and frequency of the Gm1 epitope in bacterial species in the Cape Town area is unknown. An ELISA assay for the Gm1 epitope was used to screen 17 type and 474 clinical and veterinary strains of 16 *Campylobacter*, 4 *Helicobacter* and 3 *Arcobacter* species/subspecies plus 18 *E. coli* strains isolated from diarrhetic infants. Overall, 133/491 (27.5%) of the strains were positive for Gm1. Of 92 *C. jejuni* chicken isolates tested, 28 (30.4%) were positive, as were 13 of 37 (35.2%) ostrich isolates. Positive clinical isolates, 66/222 (29.7%), of *C. jejuni* biotype 1 were obtained from 4 cases of GBS, 3 blood cultures and 59 cases of enteritis. Positive *C. jejuni* biotype 2 isolates, 32/63 (50.8%), were from 7 GBS cases, the rest from chronic diarrhea cases. One *C. coli*, one *C. mucosalis* one *H. acinonyx* and two *C. jejuni* subsp. *doylei* isolates were also positive. Thirty-two of 42 (76.2%) *H. pylori* strains tested were positive. All other bacterial isolates tested were negative. The relatively common occurrence of the Gm1 epitope in clinical and veterinary *Campylobacter* strains with which GBS is an uncommon sequelae, plus the observation that two *C. jejuni* strains isolated from GBS patients were Gm1 negative, suggests that other factors, such as genetic predisposition, are important in the development of GBS. The occurrence of the Gm1 epitope, like the Penner serotype, may simply be a marker for *Campylobacter* strains with GBS potential. The finding of the Gm1 epitope on strains of *Helicobacter pylori* is novel, and deserves further investigation.

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CB2

## Heterogeneity of *C. jejuni* from Guillain-Barré and Miller Fisher Patients.

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**Introduction** *C.* jejuni has been identified as the predominant antecedent infection in Guillain-Barré Syndrome (GBS) and Miller Fisher Syndrome (MFS). Apart from host factors, factors related to *C.jejuni* that are located in the LPS fraction may play an important role in the pathogenesis of GBS. The risk of developing GBS/MFS may be higher with specific *C.jejuni* types and it has been suggested that GBS-related *C.jejuni* are clonaly related. We set up this study to analyze the genetic variation among Dutch GBS-MFS-related *C.jejuni.* **Materials & Methods.** We collected 18 GBS or MFS-related *C.jejuni* strains from patients in The Netherlands from 1991-98, and determined the clonality or diversity among these strains by using serotyping (Penner, Lior), flaA PCR-RFLP, AFLP, PFGE and RAPD. **Results & Discussion.** Serotyping of 18 GBS/MFS-related strains revealed at least 8 different O-serotypes. *C.jejuni* O:19 was encountered in 2/18 of GBS patients. *C.jejuni* O:2, a prevailing serotype in enteritis patients, was encountered in two GBS patients and two family members with diarrhea. All patients with MFS had *C.jejuni* of different O-serotypes. In contrast to recent reports from the USA, Japan and South Africa, the overrepresentation of certain O-serotypes is certainly not a universal phenomenon. The conclusions drawn from the results of serotyping were corroborated by the compiled data of the different molecular typing methods, which demonstrated substantial heterogeneity. Clustering occured, but strains from GBS/ MFS as well as enteritis patients were present in these clusters. No clustering of GBS or MFS-related strains was found, irrespective of the method. The methods, however, may lack discriminatory power to detect the particular determinants of *C.jejuni* related to GBS/MFS. If these determinants reside in the LOS/LPS genes, then genetic analysis of genes involved in the synthesis of LPS and incorporation of sialic acid is likely to play a pivotal role in unraveling the pathogenesis of GBS/MFS.

CB3 **The LOS Biosynthesis Locus of *Campylobacter jejuni* OH4384: Identification of the Glycosyltransferases Used for the Biosynthesis of Ganglioside Mimics.** M. GILBERT, A.-M. CUNNINGHAM, M.-F. KARWASKI, J. MICHNIEWICS, Y. WU, N.M. YOUNG and W.W. WAKARCHUK. Institute for Biological Sciences, National Research Council of Canada, Ottawa, Ontario, Canada, K1A 0R6.

We have applied two strategies for the cloning of four genes responsible for the biosynthesis of the GT1a ganglioside mimic in the LOS of *Campylobacter jejuni* OH4384. We first cloned a gene encoding an α-2,3-sialyltransferase (*cst-I*) using an activity screening strategy. We then used nucleotide sequence information from the recently completed sequence from *C. jejuni* NCTC 11168 to amplify a region involved in LOS biosynthesis from *C. jejuni* OH4384. Potential glycosyltransferase genes were cloned individually, expressed in *Escherichia coli* and assayed using synthetic fluorescent oligosaccharides as acceptors. We identified genes encoding a β-1,4-*N*-acetylgalactosaminyltransferase (*cgtA*), a β-1,3-galactosyltransferase (*cgtB*) and a bifunctional sialyltransferase (*cst-II*) which transfers sialic acid to O-3 of galactose and to O-8 of a sialic acid that is linked α-2,3- to a galactose. The role of *cgtA* and *cst-II* in the synthesis of the GT1a mimic in *C. jejuni* OH4384 were confirmed by compairing their sequence and activity with corresponding homologues in two related *C. jejuni* strains that express shorter ganglioside mimics in their LOS.

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CB4 Characterization of *pgl* Mutants in an Isolate of *Campylobacter jejuni* from a Patient with Miller Fisher Syndrome

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We have recently described a gene cluster, *pglA-G*, in *Campylobacter jejuni* 81-176 (O:23/36) which encodes proteins involved in glycosylation of numerous proteins, including flagellin. We have previously shown for *pgl* mutants of 81-176, that numerous proteins, including flagellin, lose immunoreactivity with O:23 and/or O:36 antisera. Moreover, comparison of protein immunoreactivity of wildtype 81-176 and *pgl* mutants with immune sera from experimentally infected humans indicates that the glycosyl moieties are recognized in protected individuals. Although LPS from *E. coli* harbouring a subset of these genes became reactive with *C. jejuni* O:23 and O:36 antisera, mutation of these genes in 81-176 did not result in any detectable changes in LPS. We have examined the effect of *pgl* gene mutation in PG836, a *C. jejuni* strain (O:10) isolated from a patient with Miller Fisher Syndrome, an ocular variant of Guillain Barré Syndrome. PCR results indicate that PG836 contains all seven *pgl* genes. PG836 mutants in *pglA*, *pglB*, and *pglD* were constructed and analysed. Numerous proteins from *pglA*, *pglB*, and *pglD* mutants in PG836 lose reactivity with serum from the Miller Fisher patient. However, unlike 81-176, comparison of PG836 LOS cores with those of the mutants by silver staining demonstrates differences in mobility. In addition, the *pglD* mutant exhibits a decrease in motility in comparison to wildtype PG836. The biological effects of *pgl* mutations will be examined further comparing cell morphology, growth rates and mouse colonization relative to that of wildtype PG836. Preliminary results suggest that mutation of *pgl* genes in different *C. jejuni* strains results in different phenotypes and that in some strains the Pgl proteins may be involved in both LPS biosynthesis and protein glycosylation.

CB5

Multiple *N*-Acetyl neuraminic acid synthetase (*neuB*) genes in *Campylobacter jejuni*: identification and characterisation of the gene involved in sialylation of lipo-oligosaccharide

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*N*-acetyl neuraminic acid (NANA) is a common constituent of *Campylobacter jejuni* lipo-oligosaccharide (LOS). Such structures often mimic human gangliosides and are thought to be involved in the triggering of Guillain-Barré and Miller-Fisher syndromes following *C. jejuni* infection. Analysis of the *C. jejuni* NCTC 11168 genome sequence identified three putative NANA synthetase genes termed *neuB*$_1$, *neuB*$_2$ and *neuB*$_3$. Isogenic mutants were created in all three *neuB* genes and for one such mutant (*neuB*$_3$) LOS was shown to have increased mobility and no longer bound cholera toxin. Gas-chromatography mass-spectrometry and fast atom bombardment mass-spectrometry analysis of LOS from wild-type and the *neuB*$_3$ mutant strain demonstrated the lack of NANA in the latter. Expression of the *neuB*$_3$ gene in *E. coli* confirmed that NeuB$_3$ was capable of NANA biosynthesis through condensation of *N*-acetyl-D-mannosamine and phosphoenolpyruvate. Southern analysis demonstrated that the *neuB*$_3$ gene was confined to strains of *C. jejuni* with LOS containing a single NANA residue.

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CB6

***Campylobacter jejuni* (Cj) Infection and Acute Inflammatory Demyelinating Neuropathy (AIDP).** SP GORTHY, R CHAUDHRY, M BIHARI, N. SHARMA, G PEREZ-PEREZ, and P. PANIGRAHI. All India Institute of Medical Sciences (AIIMS), INDIA; Vanderbilt University and University of Maryland School of Medicine, USA.

The clinical and epidemiologic aspects of antecedent Cj infection and AIDP are ill understood and have not been studied in the Indian subcontinent. In this study, we examined the incidence of Cj infection in AIDP, and compared the severity of case presentation in seropositive and seronegative groups.

A prospective study was performed in 20 patients with AIDP, 20 matched household controls, and 20 matched hospital controls at AIIMS. Standard bacteriological and serological (IgM, IgG, and IgA ELISA) techniques were used. Serological evidence of Cj infection was noted in 7 patients with AIDP, 6 household controls (p=NS), and no hospital controls ($p<0.01$). Stool culture was negative for Cj in all patients and controls. History of antecedent illness was present in 70% of cases. Enteritis was present in only 15% of cases. Only 2/7 Cj seropositive cases had a history of enteritis. All Cj seropositive cases had a benign hospital course with no fatalities, but remained dependent for activities of daily living at time of discharge (mean hospital stay 15±2 days). In the Cj negative group, antecedent diarrheal illness was present in 7%, and upper respiratory infection in 84% of patients. All Cj negative cases had a stormy course, with 6/13 requiring assisted ventilation; 3 died. Electrophysiologic studies in 19/20 patients showed abnormalities; 80% of them could not be classified as either having axonal or demyelinating abnormality. Five patients had universal unresponsiveness. Sensory nerve studies showed 14% unresponsiveness in the Cj positive group and 53% in the Cj negative group. Cj infection could not be conclusively associated with acute AIDP in this study population.

**CB7** Immune responses in GBS patients with prior infection by clonal *Campylobacter jejuni* of Penner 0-41 serotype.

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Infection by *Campylobacter jejuni* is the commonest recognised infection world-wide preceding the development of GBS. A commonly accepted explanation for this association is the generation of cross-reactive antibodies generated against ganglioside haptenic determinants present on the LOS/LPS of some strains of organism. There are difficulties with this proposal since 30-40% of *Campylobacter*-associated GBS patients do not appear to develop anti-ganglioside antibodies. Recent studies have demonstrated that the presence of ganglioside sequences in *Campylobacter* LPS is more common than originally thought and is clearly not restricted to isolates obtained from GBS patients. There is some association between serotype and association with GBS, but this is different in different regions of the world. Recently the isolation in Cape Town of organisms with the rare Penner 0-41 serotype was strongly associated with GBS. Restriction DNA analysis suggested that these isolates were clonally related. The sera from six of the GBS patients and one with Miller Fisher syndrome from whom isolates were obtained have been examined for anti-ganglioside antibodies. Only two of the patients were found to have produced anti-GM1 antibodies, and in only one were the antibodies of high titre. Antibodies were of all Ig classes in this patient. The IgA anti-GM1 antibodies were of the IgA1 sub-class and atypical of anti-LPS antibodies. The MFS patient's serum contained anti-GD3 antibodies.TLC immuno-overlay indicated the presence of low levels of anti-GD1b antibodies in 3 patients. ELISA determination of anti-*Campylobacter* antibodies using antigen derived from European strains indicated sero-conversion in 4/7 patients. These results suggest that the production of anti-ganglioside antibodies is not obligatory for the development of GBS following *Campylobacter* infection.

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**CB8** Development of a Rapid Assay for $GM_1$ Epitope Screening of *Campylobacter jejuni*

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Molecular mimicry between epitopes on *Campylobacter jejuni* lipopolysaccharides (LPSs) and peripheral nerve gangliosides, particularly $GM_1$ ganglioside, has been proposed as a mechanism for induction of antiganglioside antibodies. These antibodies are considered to play a role in *C. jejuni*–associated Guillain-Barré syndrome (GBS). As methods used for analysis of LPS are both labor intensive and time consuming, which does not allow for the screening of large numbers of strains, we developed a rapid $GM_1$ epitope screening procedure. Biomass harvested from two plates of confluent growth was sufficient to isolate LPS using a novel phenol-water and ether extraction procedure. Extracts of LPS were tested for reaction with three ligands: anti-$GM_1$ antibodies, cholera toxin (a $GM_1$ ligand) and peanut agglutinin (a Gal$\beta$1$\rightarrow$3GalNAc ligand). First, the assay was validated by comparing results obtained using mini-phenol-water extracted LPSs from ten *C. jejuni* strains with data from previous binding studies, using purified LPS. Second, LPS extracts from 5/7 (71%) *C. jejuni* GBS isolates and 2/3 (67%) *C. jejuni* culture collection strains were positive for binding with two or more anti-$GM_1$ ligands. Subsequently, 12/59 (20%) of *C. jejuni* serotype reference strains tested demonstrated binding with two or more anti-$GM_1$ reagents, suggesting $GM_1$ mimicry in these strains. Of these strains, four serotypes have not previously been found in association with GBS. Overall, the assay system was reliable, efficient and reproducible and it could be adapted for large epidemiological studies.

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CB10

**_Campylobacter jejuni_ lipopolysaccharides from Guillain-Barré and Miller Fisher patients induce anti-GM1 and anti-GQ1b antibodies in rabbits**

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Anti-ganglioside antibodies in patients with Guillain-Barré syndrome (GBS) and Miller Fisher syndrome (MFS) are thought to be induced by molecular mimicry between *Campylobacter jejuni* lipopolysaccharides (LPS) and gangliosides. We used purified LPS fractions from 5 *Campylobacter* strains to induce anti-ganglioside responses in rabbits.Two strains were isolated from GBS patients with anti-GM1 and anti-asialoGM1 antibodies. Two other strains were isolated from MFS patients with anti-GQ1b antibodies. The Penner O:3 serostrain which lacks ganglioside-like epitopes was used as control. Immunization of New Zealand White rabbits with LPS in complete Freund's adjuvant resulted in high titer IgG anti-LPS and anti-ganglioside antibodies and lower titer IgM antibodies. The animals that received injections with LPS from GBS-associated strains developed anti-GM1 and anti-asialo-GM1 antibodies. Animals that were injected with LPS from MFS patients had anti-GQ1b antibodies. The rabbits that were injected with Pen O:3 LPS had a strong anti-LPS response but no anti-ganglioside reactivity was observed. One animal that was immunized with a GBS associated strain developed clinical symptoms. Our results demonstrate that an immune response against *C. jejuni* LPS can induce anti-ganglioside antibodies and that the LPS structure determines the specificity of the immune response.

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CB12

**LPS structure determines anti-ganglioside antibody specificity and clinical features in patients with Guillain-Barré and Miller Fisher syndrome**

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In patients with the Guillain-Barré syndrome (GBS) and Miller Fisher syndrome (MFS), anti-ganglioside antibodies are thought to be induced by a preceding *Campylobacter* infection through molecular mimicry between *C. jejuni* lipopolysaccharides (LPS) and neural gangliosides. We characterized the purified LPS fraction of 18 Campylobacter strains isolated from GBS or MFS cases with a panel of GM1-positive and GQ1b-positive serum samples. LPS from 17/18 strains showed reactivity with GM1 and/or GQ1b positive serum samples, confirming the frequent presence of ganglioside-like epitopes in GBS and MFS associated strains. All patients but one had antibody reactivity against the LPS fraction from the strain isolated from himself/herself. When patients were tested on a panel of gangliosides, anti-GM1 antibodies were associated with the presence of a GM1-like epitope in the LPS. The presence of a GQ1b-like epitope in the LPS fraction correlated with serum reactivity against GQ1b and the presence of oculomotor symptoms. Our results demonstrate that the immunological reactivity of the LPS fraction determines the anti-ganglioside specificity and clinical features in post-*Campylobacter* neuropathies.

CB13

## Genetic characterization of *Campylobacter jejuni* O:41 isolates in relation with Guillain Barré Syndrome.

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*Campylobacter jejuni* serotype O:41 strains have been found in association with Guillain Barré syndrome (GBS) patients in the Cape Town area of South Africa. *C. jejuni* serotype O:41 strains which were collected over a 17 year period, were isolated from GBS patients, enteritis patients, and from an ostrich with fatal enteritis. These strains were genetically characterized by flagellin gene restriction length polymorphism (RFLP) analysis, amplified fragment length polymorphism (AFLP) analysis, and RFLP analysis of the sugar transferase genes involved in LPS biosynthesis. Although these *Campylobacter* strains displayed variation in the expression of the GM1 epitope, which is thought to be involved in the pathogenesis of GBS, when analyzed by molecular techniques, all strains were genetically indistinguishable. No differences were detected between recent and historic isolates, or strains that carry or did not carry the GM1 epitope. The flagellin genotype of the O:41 isolates was identical to the genotype of O:19 strains, the serotype most commonly associated with GBS in other parts of the world. However, differences were detected in the LPS gene locus of these two serogroups. The overall genomic similarity of serogroups O:19 and O:41 was 60% as determined by AFLP. *C. jejuni* serotype O:41 strains isolated in the Cape Town area are clonal, genetically stable, and are persistent in the environment.

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CB14

## Surface ganglioside epitopes on animal isolates of Campylobacter spp.

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Campylobacter strains with Gm1 ganglioside are being thought to contribute to the pathogenesis of Guillain-Barre syndrome. In this study, to detect animal Campylobacter strains that bind cholera toxin as an indicator of Gm1 ganglioside on their surface, a rapid screening assay was used. In the assay, boiled bacterial suspensions were incubated with cholera B subunit. Campylobacter strains with Gm1 inhibited the binding of cholera B subunit to Gm1 in a microtiter enzyme-linked immunosorbent assay. Nineteen (21.1%) of 90 Campylobacter strains isolated from animals were found to bind B subunit. Fourteen of 60 C. jejuni and 5 of 18 C.coli strains were positive in the microtiter assay. Positive C.jejuni strains were from chickens, sheep, dogs and cattle. Other positive C.coli strains were isolated from chickens and sheep. No positive strains were identified among canine C.upsaliensis, ovine C. fetus subsp. fetus and bovine C.hyointestinalis isolates. Although, Gm1 is not the only ganglioside which bind to cholera toxin, the presence of cholera toxin-binding ability in many C.jejuni and C.coli isolates showed that this epitope is relatively common among animal strains. If Gm1 ganglioside plays a role in the pathogenesis of Guillain-Barre syndrome, animals may be considered as a potential source.

CB15

**_Campylobacter jejuni_ and Guillain-Barre Syndrome in Mexico City: A Prospective Culture Based Analysis of O Serotypes Associated with Disease.** I. NACHAMKIN, P. ARZATE, L. FLORES, A. GONZALEZ, P. RODRIQUEZ, M. NICHOLSON, C. LOBATO, T.W. HO, A.K. ASBURY, J.W. GRIFFIN, and G.M. McKHANN. U. of Pennsylvania, Philadelphia; Instituto Nacional Pediatria, Mexico City; CDC, Atlanta, GA., Johns Hopkins Univ. , Baltimore

Guillain-Barre Syndrome (GBS) occurs worldwide and the association with prior *Campylobacter jejuni* infection is well established. However, the epidemiology of *Campylobacter* associated GBS is poorly defined in many geographic locations. In 1997, we began a prospective study of different forms of GBS at a children's hospital in Mexico City. Stool samples from all patients admitted to the Neurology Service for evaluation of GBS were cultured for *Campylobacter* using Preston enrichment broth and CCDA selective media. Fifty-three patients with GBS were cultured, of which 36 were male and 17 female and ranged in age from 21 months to 17 years. Six GBS patients (11.3%) were culture positive for *C. jejuni*. The isolates belonged to three O serotypes, O:19 (50%), O:41 (16.6%), O:37,16 (16.6%), and one was not-typable using a panel of 24- O typing sera. Twenty-four control isolates obtained from patients with diarrhea were also serotyped using the same O typing sera. There were 12 different O serotypes among the control isolates, 5/24 (20.8%) were O:19. The proportion of O:19 isolates in GBS and control populations were not significantly different (p=0.3). While the number of patients analyzed in this study are small, these preliminary results suggest the high rate of O:19 isolates in GBS patients may be a function of the rather high prevalence of this serotype circulating among the general population.

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**Prospective study of IgG antibodies to _Campylobacter jejuni_ and GM1 antibodies in a patient with Guillain-Barré syndrome.** CB16

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*Campylobacter jejuni* is a common cause of gastroenteritis in Chile, chicken carcasses and poultry products obtained at outlet establishments are frequently contaminated with this bacteria (45%). On the other hand reports from Chilean neurologists indicate that GBS neuropathy is increasing in the last years in our country.
Here we report the case of a patient (male 65) that underwent two severe episodes of GBS (Nov'97 and Nov'98) that require prolonged mechanic ventilation. IgG antibodies to *C. jejuni* and GM1ganglioside were analyzed by an "in house" ELISA. Results are presented as OD readings. The cutoff values for *C. jejuni* and GM1 assays were calculated as 0.22 and 0.1 respectively in Chilean asymptomatic population. *C. jejuni* was not isolated from fecal samples, which was probably related to the administration of antibiotics prior that the GBS symptoms were apparent.

| | **EIA titers to *C. jejuni* and GM1** | | | | | | |
|---|---|---|---|---|---|---|---|
| **Follow-up** | **T0** | **T1** | **T2** | **T3** | **T4** | **T5** | **T6** |
| ***C. jejuni*** | 1.905 | 1.139 | 0.866 | 1.077 | 1.454 | 0.40 | 0.32 |
| **GM1** | 0.831 | 0.08 | 0.125 | 0.395 | 0.389 | 0.05 | 0.05 |

As observed above, IgG antibodies to *C. jejuni* increases significantly on Nov97 (T0) and a year later Nov98 (T4). Rises of IgG antibodies to GM1 were also observed in these occasions. This is the first report of a patient with two GBS episodes in our country in which *C. jejuni* was apparently involved.

CD1

Identification and differentiation of thermophilic *Campylobacter* spp. isolated from animals by phenotyping and genotyping.

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Traditional phenotyping for species identification of thermophilic *Campylobacter* spp. is cumbersome and sometimes gives incorrect and intermediate results. Many laboratories only identify isolated campylobacters at the genus level. For epidemiological studies and for identification of sources of human infection, subtyping below the species level is performed. However, before subtyping, a reliable species identification should be carried out.

The source of human infection is often looked for among food animals, especially poultry. Also other animal species, including wild animals, have been implicated or suspected. Therefore, we performed a study on 100 isolates of campylobacters obtained from different animal species. We wanted to evaluate the applicability of a PCR-REA, and compare with traditional phenotyping for identification and differentiation between the thermophilic *C. jejuni*, *C. coli*, *C. lari*, and *C. upsaliensis*. The campylobacters were isolated from cattle, sheep, swine, wild mammals and wild birds. A fragment of the 23S rRNA gene was specifically amplified from the thermophilic campylobacters by PCR, and digestion by two restriction enzymes allowed differentiation between the four *Campylobacter* spp. Both pheno- and genotyping identified 64 isolates as *C. jejuni*, 12 as *C. coli*, 2 as *C. lari* and 4 as other (non-thermophilic) campylobacters. For the majority of the remaining isolates, no specific species identification could be done by phenotyping, due to weak reactions or conflicting test results. By PCR-REA 5 of these were identified as *C. jejuni*, 9 as *C. coli*, 1 as *C. lari*, and 3 were non-thermophilic campylobacters.

The PCR-REA proved very valuable, and could be used as a complement or alone for species identification of animal isolates of thermophilic *Campylobacter* spp.

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CD2

## The effect of oxygen, carbon dioxide and hydrogen on the growth of *Campylobacter* species.

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Preston Public Health Laboratory, Preston, UK.

*Campylobacter* species are microaerobic to anaerobic with a respiratory metabolism and hydrogen is required or stimulates growth. Previous studies on the gaseous requirements of *Campylobacter* species have found that carbon dioxide can increase recovery and survival leading to the suggestion that they are capnophilic rather than microaerobic.

The Modular Atmosphere Controlled System with Variable Atmosphere (MACS VA500) is an incubator within which the concentrations of oxygen, carbon dioxide and hydrogen can be altered in a balance of nitrogen. The aim of this study was to determine the effect of varying the concentrations of these gases on the growth of a range of *Campylobacter* species.

Test strains were inoculated onto nutrient and blood agar and incubated in the test gaseous environment at 37°C for 72 hours. Duplicate sets of culture plates were incubated in a control atmosphere of 5%$O_2$, 5%$CO_2$, 5%$H_2$ in $N_2$. Growth was determined by enumeration of colonies and measuring colony sizes.

It was found that 5% $O_2$ in $N_2$ and 5% $H_2$ in $N_2$ were insufficient to support the growth of the strains tested with the exception of *C.mucosalis*. Five percent $CO_2$ in $N_2$ allowed the growth of all test strains with little difference in $\log_{10}$ recovery in test and control conditions. Colonies were smaller in 5% $CO_2$ than the control atmosphere. *C. jejuni* and *C. coli* grew well in most atmospheres containing at least 5% $CO_2$ but grew equally well in a mixture of 5%$O_2$, 5%$H_2$ in a balance of $N_2$. Mixtures of $O_2$, $CO_2$ and $H_2$ supported better growth than individual gases in $N_2$. Implications for isolation of *Campylobacter* species will be presented.

NOTES

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CD3

**Comparison of *Campy*-SELeCT™ to Gold Standard Methodology for Rapid Detection and Enumeration of *Campylobacter* in Food and Clinical Stool Specimens.**

**D.M. ROLLINS, L.H. LEE, S.A. CARROLL, S.W. Walz and S.W. JOSEPH. Naval Medical Research Center, Forest Glen, MD and University of Maryland, College Park, MD**

***Campy*-SELeCT™ (*Salmonella*, *E. coli* O157:H7, *Listeria*, and *Campylobacter* Test) was developed in our laboratories as a means of enumerating bioloads on naturally contaminated poultry and food. We have also adapted the *Campy*-SELeCT™ for use with human stool specimens. Processing and selective isolation is followed by identification and enumeration of target organisms either visually or with a colony lift immunoassay (CLI). The method is rapid, sensitive, specific and less labor and resource intensive than standard isolation, quantification and biochemical identification for the accurate detection and enumeration of *Campylobacter jejuni/coli* across a wide range of bioloads (1 – $10^7$ CFU).**

**The *Campy*-SELeCT™ has outperformed the Most Probable Number technique (MPN), which is the gold standard for enumeration of bacteria in foods. The *Campy*-SELeCT™ was also evaluated using stool specimens from naturally-acquired disease episodes occurring during a military field exercise in Thailand and with stools from volunteers experimentally-infected with *C. jejuni*. In both settings, the *Campy*-SELeCT™ was comparable or superior to the gold standard methods of the study.**

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CD4

**Campylobacter infection in neonate and puppy: a genotypically proven relationship**

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A three-week old child was presented at the Children's Hospital with fever. She had been well until 5 days before admission, when she started vomiting, produced mucoid stools and became lethargic and irritable. Sepsis evaluation was done and after cerebrospinal fluid appeared normal, treatment with amoxycillin-clavulanic acid and gentamicin was initiated. The clinical situation improved within 12 hours. After 5 days, the blood culture became positive for Campylobacter. A fecal sample was also positive for Campylobacter. The therapy was changed into erythromycin. The child was discharged from the hospital after 10 days of hospitalisation.
Six days prior to the hospitalisation of the child, a Labrador retriever puppy arrived in the family. This dog had soft faeces and lost appetite from the day of arrival and a serious diarrhoea started 6 days after hospitalisation of the child. Twelve days later, a faecal sample was found positive for Campylobacter. The Campylobacter isolates from the child (blood and faeces) and the dog were genotyped by AFLP which was shown recently to have a high discriminatory power. The three isolates showed the same *Campylobacter jejuni* genotype. In progress is the sampling of the puppies from the same litter to elucidate the initial infection of the puppy. This case represents to our knowledge the first bacteriological proven relationship between Campylobacter infection in child and dog within the same family.

CD5 **Evaluation of the VIDAS *Campylobacter* Assay for direct detection of *Campylobacter* in broiler caecal material**

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Conventional methodologies for detection of Campylobacter require 2-3 days, including selective enrichment, plating and presumptive identification by microscope. The VIDAS *Campylobacter* (CAM) Assay is intended for use in the Vitek Immuno Diagnostic Assay System (VIDAS; bioMérieux sa, Marcy-L'Etoile, France) as an automated qualitative enzyme-linked fluorescent immunoassay (ELFA) for a rapid (within 2 h) and specific detection of *Campylobacter* in food samples, after an enrichment procedure.
A pilot study was carried out to evaluate the VIDAS CAM Assay for direct detection of *Campylobacter* in broiler caecal material, without enrichment. Samples were obtained from control and infected birds within challenge experiments, therefore *Campylobacter* was known to be present in 79 of a total of 115 samples (69 %). *Campylobacter* was detected by conventional plating on CCDA in all 45 fresh *Campylobacter*-positive samples, but failed in 14 out of 34 samples that had been stored for 6 weeks at -40°C. VIDAS CAM correctly detected *Campylobacter* in all the 34 stored samples and in all-but-one of the 45 fresh samples. Overall, 1 (0.9 %) false-negative and 10 (9 %) false-positive results were observed with the VIDAS CAM Assay. The results from this pilot study indicate that the VIDAS CAM Assay can be used for direct detection of *Campylobacter* in fresh as well as stored caecal material. Optimization of the VIDAS caecal sample preparation protocol is currently carried out to reduce the number of false-positive results. The adjusted protocol will be validated in a second evaluation study, and results will be presented.

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CD6 **Characterization of a group of urease-positive thermophilic Campylobacters from stool and blood.** Leta O. Helsel[1], M.A. Nicholson[1], C. Fitzgerald[1], D. Lindquist[2], P.I.. Fields[1]; [1]Centers for Disease Control and Prevention, Atlanta, GA and [2]California Department of Health, Berkeley, CA

Most species of *Campylobacter* do not produce urease; however, urease- positive thermophilic Campylobacters (UPTCs) are being increasingly recognized. Many UPTCs are considered to be variants of *C. lari*. In addition, a urease-positive biovar of *C. sputorum* has recently been described. UPTCs have been isolated from a number of different animal reservoirs and the aquatic environment. They are not routinely isolated from humans; to date, only four human isolates of urease-positive *C. lari* have been reported. We have characterized 7 human isolates of UPTC, 5 from stool and 2 from blood, obtained from the reference collection at the CDC. They were isolated between 1988 and 1998. Two stool isolates were from individuals with gastroenteritis, 2 stool isolates were from asymptomatic individuals, and one blood isolate was from an individual with underlying disease. The clinical diagnosis is unknown for the remaining two isolates. Biochemically, these isolates were similar to *C. lari* except that they were urease-positive and sensitive to nalidixic acid. They possessed the cellular fatty acid profile that is typical of several *Campylobacter* spp., including *C. lari*. They were positive in a *Campylobacter* genus-specific PCR based on 16S rRNA sequence, but negative in a *C. lari* 16S PCR, as well as in several other species-specific PCRs. 16S rRNA sequences from 6 of the isolates were very similar to each other and similar to a 16S rRNA sequence from a UPTC isolate that was in the GenBank database. The 16S rRNA sequence from the UPTC isolates were distinct from the two *C. lari* sequences in the database and from two *C. lari* isolates that were sequenced at the CDC, and were more closely related to the *C. jejuni* 16S rRNA sequences. DNA-DNA hybridization studies are underway to determine the true phylogenetic position of the UPTCs.

**Evaluation of a panel of PCR assays for the identification of *Campylobacter* spp.** CD7

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The current gold standard for identification of *Campylobacter* spp is biochemical identification and cellular fatty acid (CFA) analysis. However, they are negative in many biochemical tests and are often differentiated on the basis of a single biochemical test. CFA analysis cannot differentiate several *Campylobacter* spp., and hippuricase negative *C. jejuni* can not be differentiated from *C. coli* in the current scheme. In an attempt to improve and simplify the identification of *Campylobacter* spp, we evaluated a panel of PCR assays for the identification and differentiation of the species. The PCR assays tested were: i) a genus specific PCR and species specific PCRs for *C. lari, C. upsaliensis, C. fetus, C. hyointestinalis*, and *C. helveticus* that were based on 16s rRNA sequences (Linton *et al.* Res. Microbiol. 147:707-718, 1996); ii) a PCR that differentiated *C. jejuni* and *C. coli* based on the *ceuE* gene (Gonzalez *et al.* J. Clin. Microbiol. 35:759-763, 1997); iii) a PCR that detected *hipO* (Linton *et al.* J. Clin. Microbiol. 35:2568-2572, 1997); and iv) primers based on 16S rRNA sequence developed at the CDC for the detection of *C. sputorum*. One hundred-forty *Campylobacter*, *Campylobacter*-like organisms (CLO), *Helicobacter*, and *Arcobacter* isolates as well as a panel of unrelated bacterial species were tested in the PCRs. All 11 *C. sputorum* isolates tested, representing 2 of the 3 biovars, were positive in the *C. sputorum* specific PCR; the PCR was negative for all other isolates tested. For the most part, the published PCRs performed as expected. Thirty-three hippuricase negative *C. jejuni/coli* isolates were tested; all were identified as either *C. jejuni* (6 isolates) or *C. coli* (27 isolates) with the *ceuE* and *hipO* PCRs. In our hands, the *Campylobacter* genus PCR also detected *Arcobacter* spp. The *ceuE* PCR for the detection of *C. jejuni* did not detect 4 of 5 C. *jejuni* ssp *doylei* isolates. This panel of PCRs should be useful as the basis for the molecular detection of *Campylobacter* spp.

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IDENTIFICATION OF CAMPYLOBACTER JEJUNI AND C. COLI FROM ENVIREMENTAL SAMPLES IN POULTRY FARMS IN GERMANY CD8

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Campyolbacter jejuni and C. coli are difficult and laborious to isolate and cultivate from food or environmental samples. In order to investigate for the presence of Campylobacter spp. swab, water and faeces samples were taken from three poultry flocks. The identification rate from water samples examined with the aid of cultural microbiological methods was 6,6% positive for Campylobacter. The results from RFLP-PCR based on fla A and fla B genes schows a higher identification rate of Campylobacter jejuni / C. coli (4,1 to 23%) in water from laying flicks. The swabs sampled – 30,8% were positive for Campylobacter with ral methods and 44,1% with RFLP-PCR .

CD9 **_Campylobacter upsaliensis_ in Canada (1985-1998)** NOTES

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*Campylobacter upsaliensis,* first isolated in 1983 from healthy and diahrreic dogs, has since been isolated from humans, and is recognized as being associated with a spectrum of illnesses, including gastroenteritis and bacteremia. The National Laboratory for Enteric Pathogens (NLEP) has provided identification of Campylobacter isolates to the species level. In addition, surveillance data were obtained from reports provided by the Provincial Laboratories of Public Health in Canada. Phenotypic identification was performed on all isolates, and this was complimented with cellular fatty acid (CFA) composition analysis, PCR ribotyping, and 16S rRNA sequencing. During the period 1985 to date, a total of 345 clinical isolates of *Campylobacter*-like organisms were submitted to the NLEP for identification. Of these, 78 were identified as *C. upsaliensis*. Based on available data, of the 76 human submissions, 70 % were isolated from stools, and 2.6 % from blood. Among the isolates, there were 2 family related cases. Additionally, isolates were recovered from the stools and blood of a newborn child, in a household where *C. upsaliensis* was also recovered from family pets, (cat and dog). These data suggest that person-to-person and animal-to-human transmission may have occurred. Identification of *C. upsaliensis* isolates at the NLEP, along with surveillance data, provides valuable epidemiological information related to incidence, risk factors, trends and transmission of this emerging pathogen.

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CD10 **Serotyping and Biotyping of *Campylobacter* from Canadian and International Sources between 1983 and 1998.** D.L. WOODWARD, Y.D. YASCHUK, L.J. PRICE, A. MOTERASSED, J. MOSES, W. JOHNSON , and F.G. RODGERS. The Canadian Science Center for Human and Animal Health, Bureau of Microbiology, National Laboratory for Enteric Pathogens, Health Canada, Winnipeg, Manitoba, R3E 3R2, Canada

Campylobacters are one of the most common bacterial agents of human diarrheal disease. A total of 20 species and subspecies are recognized within the genus *Campylobacter* and have been associated with human and/or animal disease. Laboratory surveillance activities have played a fundamental role in support of epidemiological investigations involved in tracing infections and in studying emerging trends related to these organisms. Data on Campylobacters were obtained from reports provided by Provincial Laboratories of Public Health. The National Laboratory for Enteric Pathogens (NLEP) has provided support for species identification, serotyping and biotyping. In Canada, the number of laboratory reported cases of campylobacteriosis dramatically increased from 2,915 in 1983 to 13,639 in 1993. The number of isolations of Campylobacter remain high and was 12,359 in 1998. A serotyping scheme developed at NLEP for the detection of heat labile antigens of *C. jejuni, C. coli* and *C. lari* recognizes 130 serotypes. During the period of 1983-1998, a total of 6,349 isolates comprising 5,042 from human, 1,254 from non-human and 53 from non-stated sources originating both nationally and internationally were serotyped at NLEP. Identification studies on 5,138 isolates yielded 4,045 *C.jejuni*, 1,056 *C.coli*, and 37 *C. lari*. Biotyping and serotyping investigations provided valuable epidemiological information on the incidence, trends and risk factors related to outbreaks and clinical cases. As a risk management tool this can be used to increase public awareness through education and assist in the development of intervention strategies for prevention and control measures.

## Identification of Ciprofloxacin Resistant *Campylobacter jejuni* by means of a Fluorogenic PCR Assay

CD11

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Fluoroquinolones, tetracyclines and erythromycin are the antibiotics commonly used to fight *Campylobacter jejuni* infection. *C. jejuni* strains resistant to high levels (defined here as an MIC $\geq$ 16 μg/ml) of ciprofloxacin have been predominantly characterized with a T→C transition in codon 86 of *gyrA*, a gene which encodes one subunit of DNA gyrase, and is a target for fluoroquinolone antibiotics. This study establishes a rapid method for identifying *C. jejuni* strains with high level ciprofloxacin resistance, and should prove useful for monitoring the development of resistance to this fluoroquinolone. Our assay uses a PCR-based Taqman technology to identify, and discriminate between wild-type *C. jejuni* and strains carrying the codon 86 point mutation. Sequencing data of the quinolone resistance determining region (QRDR) of each of our *Campylobacter* isolates was used to design primers and Taqman probes for the assay. Optimization of the assay parameters was performed using pure DNA from human and animal isolates of *Campylobacter*. Our results indicate the assay is *C. jejuni* specific and it detects femtogram levels of DNA. The assay uses real-time detection, eliminating the need for gel electrophoresis. In addition, the sequence data compiled on *Campylobacter* QRDRs support the use of *gyrA* sequencing as a method for speciating *Campylobacter* isolates.

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## Campylobacteria Research in Lagos, Nigeria

CD12

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Campylobacteria research worldwide has witnessed tremendous growth since the first report of isolation of *Campylobacter fetus* in 1913. In Lagos, Nigeria the first report was in 1983 on the association of *C. jejuni* with diarrhoea. Our research theme include isolation, characterisation, clinical, immunological and molecular epidemiology of *C. jejuni* and *C. coli*. Studies on *H. pylori* began in 1989 and currently we are looking at clinical, microbiological and epidemiological studies on the organism and the associated diseases. In addition to these studies we are using bioinformatics and medical informatics tools to unravel the emerging understanding of the campylobacteria. We have initiated studies on the prevalence of *Arcobacter* species in animals and humans. One landmark in our studies is the description of a medium termed Butlzer-type medium which has enabled the routine isolation of *C. jejuni* and *C. coli*. At the close of this millennium our research has helped to improve the understanding of the campylobacteria in Nigeria.

CD13 **Chemotaxis of *Campylobacter jejuni* after Prolonged Incubation in Aqueous Environment Is Dependent on Temperature**

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In a previous study[1] it was shown that although growth was not possible, *C. jejuni* was able to perform chemotaxis at environmental temperatures. In the environment, campylobacters are shed in water and survive for several weeks. Therefore, in this study, we determined the ability of *C. jejuni* to perform chemotaxis after several weeks of incubation in watery environments. After an incubation period of three weeks at 4°C, chemotaxis towards formate was still observed. *C. jejuni* stored at 10°C and higher temperatures, however, lost the ability to move within ten days, although plate counts were still at 50% of the situation at $t_0$. It was concluded that, dependent on temperature, cells lose the capacity to perform chemotaxis, long before they lose their culturability.

[1] Physiological activity of *Campylobacter jejuni* far below the minimal growth temperature. 1998. Wilma C. Hazeleger, Jeroen A. Wouters, Frank M. Rombouts and Tjakko Abee. Appl. Environ. Microbiol. 64, 3917-3922.

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CD14 **Comparison of phenotypic and genotypic methods for identification and differentiation of *Campylobacter fetus* subspecies**

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Strains of *Campylobacter fetus* may be assigned to one of two subspecies, *C. fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis*. Accurate differentiation of these taxa (and discrimination from closely related organisms such as arcobacters that also occur in the reproductive tract) is vital in veterinary microbiology, since subsp. *venerealis* causes an insidious form of infectious infertility in important food animals including cattle and sheep. Several methods have been described for identifying the two *C. fetus* subspecies. We present here an evaluation of their efficacy. 31 strains were characterized by standardized phenotypic tests, PFGE-DNA profiling and a multiplex PCR assay. 22/31 strains were also characterized by 16S rRNA gene sequencing. Results from phenotypic- and PFGE methods were evaluated by probabilistic- and cluster analytical methods respectively using widely available PC software. The subspecies identification suggested for 29/31 strains using phenotypic, PCR- and PFGE-based methods was entirely consistent. However, one strain identified as subsp. *venerealis* by phenotypic testing was considered subsp. *fetus* by the genetic analyses; another strain identified as subsp. *venerealis* by PCR was considered to be subsp. *fetus* with phenotypic and PFGE methods. 16S rRNA gene sequence analysis emphasized the close relationship between the subspecies, since only the subsp. *fetus* type strain sequence differed from all other strains studied by a single base substitution. The overall agreement among the phenotypic, PCR- and PFGE-based methods suggest them to be effective tools for discriminating *C. fetus* subspecies, but there remains a need for information concerning the basis for the taxonomic division of the species. The relative merits in terms of cost, ease of use, and accuracy of the tests examined will be discussed.

CD15

**Identification and discrimination of *Campylobacter* species by means of 16S rRNA gene PCR and RFLP analysis; evidence for 16S rRNA gene identity in *C. jejuni* and *C. coli*.**

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Molecular methods are increasingly described as a means for identification of *Campylobacter*. Genus-specific primers (C412F and 1228R) based on the 16S rRNA gene have been published previously, and were used here to generate an ~800 bp amplicon from each of the 14 known *Campylobacter* type strains. Ten restriction enzymes were tested for their ability to generate unique digestion profiles from each species. *Dde*I was found to be the most discriminatory of the enzymes tested, although an additional enzyme (*Spe*I) was required for differentiation betwen *C. jejuni* and *C. lari*, and was also required for differentiation of *C. gracilis* from *C. showae*/ *C. rectus*. The latter pair was not differentiated using any of the 10 restriction enzymes, and neither were *C. fetus*/ *C. hyointestinalis*. However, by using primer C412F with a universal primer (1492R) to generate an amplicon of increased (~1100 bp) size, differentiation was possible between *C. fetus* and *C. hyointestinalis* using *Xho*I. Primers C412F and 1492R were subsequently tested on ~300 strains, including multiple field strains for most species. Field strains were found to generate the same *Dde*I, *Spe*I, and *Xho*I profiles as their representative type strain, with two notable exceptions. C. *hyointestinalis* field strains generated two additional (but similar) profiles to the type profile. More significantly, 29/30 *C. coli* strains generated the *C. jejuni* type profile, with only one other strain generating the *C. coli* type profile. Further investigation showed *C. coli* field strains to share 16S rRNA gene sequences identical to *C. jejuni* field and type strains. Overall, this method shows promise for rapid and accurate discrimination of most *Campylobacter* species.

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CD16

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**Introduction:** Clinically, *Campylobacter* infections range from asymptomatic to severe illness and the importance of *Campylobacter* isolated from stool samples is often debated. Lactoferrin is released from activated neutrophils and demonstration of lactoferrin in fecal samples is closely related to intestinal inflammation. **The aim** of this study was to measure fecal lactoferrin levels in stools from patients with *Campylobacter* infection to estimate the degree of intestinal inflammation. **Materials and methods:** For isolation of *Campylobacter* species fecal samples were grown on selective media at 42°C and at 37°C using a filter technique. Lactoferrin was measured by ELISA technique. **Results:** The fecal lactoferrin levels were < 600 pg/ml in 20 normal young persons. Thus, a lactoferrin level > 600 pg/ml was regarded increased. Increased fecal lactoferrin levels were found in 37% of 46 patients with gastroenteritis and no enteric pathogens found in the stool. Increased fecal lactoferrin in patients with *C. jejuni* as the only enteric pathogen was 68% of 25 patients, with *C. coli* 20% of 5 patients, with *C. upsaliensis* 71% of 7 patients and with *C. concisus* 49% of 37 patients. The fecal lactoferrin level was increased in 69% of 16 patients with *Salmonella* or *Shigella* species. **Discussion:** No significant differences was seen in the number of patients with increased fecal lactoferrin between patients with and without enteric pathogens. The strongest trends were found for *C. jejuni* ($p = 0.07$) and *Salmonella/Shigella* species ($p = 0.1$). The lactoferrin levels in fecal samples from patients with gastroenteritis are not sufficient to detect bacterial intestinal infections but may predict the severity of the intestinal infection.

**CD17** Clinical outcome in two groups of *Campylobacter concisus* based on differences in high molecular mass proteins

**NOTES**

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*C. concisus* is regularly found in faeces from patients with gastrointestinal disorders. We conducted a retrospective study of 98 C. concisus positive patients found by routine examination of stool samples. The patient group consisted of 82 adults (mean age 50 years, range 19-91) and 16 children (mean age 5 years, range 1-10). Clinical data was obtained from the patients medical records.
Isolation of *C. concisus* was performed using a filter technique on blood agar plates and incubated for 24-48 hours at 37°C under microaerobic conditions. *C. concisus* was identified as motile, Gram negative rods and by testing the biochemical characteristics and protein profiles.
We assigned *C. concisus* into two broad groups by differences in protein profiles: group 1 (15% of the strains) resembled the *C. concisus* type strain, whereas group 2 (85% of the strains) had additional bands in the high molecular mass range and around 31 kDa. *C. concisus* was the only suspected pathogen in 73% of the patients; the majority (70%) of whom were adult immunocompromised patients (HIV, cancer, IBD and organ transplants).
The *C. concisus* group 2 was the only strain to infect immunocompetent and paediatric patients. Symptoms found were diarrhoea (96%), dyspepsia (77%) and mild fever (41%).

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**CD18** **Differentiation of Thermophilic Campylobacters at the Species Level by the use of a Fluorescent *In Situ* Hybridization Technique**

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Isolation and identification of the closely related thermophilic *Campylobacter* species can be both laborious and time-consuming. Traditionally, the differentiation between species within this group of bacteria relies on phenotypical tests, but for some isolates, the final identification can be difficult due to strain variations. In order to circumvent these problems, we have established a fast and reliable detection method based on a fluorescent *in situ* hybridization (FISH) identification technique using fluorescence-labeled species-specific oligonucleotide probes targeting the highly conserved ribosomal genes. The small size of the fluorescence-labeled oligonucleotide probes makes it possible to hybridize directly on formalin-fixed, whole bacterial cells, and as the ribosomal genes are highly expressed within each cell, the hybridization result can be viewed directly by epi-fluorescence microscopy without further amplification steps.
By sequencing and aligning ribosomal gene sequences from different *Campylobacter* species, a set of five probes was selected and tested for their ability to hybridize *in situ*. Because of the close genetic relationship between the species it was not possible to find a single specific probe for each thermophilic species, but when the probes were used in combination, a general hybridization pattern could be found for each thermophilic *Campylobacter* species. The specificity of the probes has been evaluated by whole-cell hybridization to a large number of *Campylobacter* reference strains and *Campylobacter*-like organisms as well as clinical isolates. With the probe combination, we have been able to identify the following reference strains: 40/41 *C.jejuni* strains, 17/19 *C.coli* strains, 3/4 *C.lari* and 4/4 *C.upsaliensis*. No match was found against 4/4 *Arcobacter* species. The probes were also tested on field isolates. Out of 264 tested isolates, 190 were typed as *C.jejuni*, 50 isolates as *C.coli*, 12 isolates as *C.lari* and 12 isolates as *C.upsaliensis*. Ninety-one different enterobacterial species served as negative controls without any reaction.

NOTES

## A Large-scale Survey of *Campylobacter* In Human Gastroenteritis Using PCR For Screening And PCR-ELISA For Identification

CD19

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We present data from a large-scale surveillance of *Campylobacter* incidence in man using PCR-based methodology and demonstrate the utility of this approach for investigation of the incidence and epidemiology of the full spectrum of enteropathogenic campylobacters.

Over a two-year period DNA was extracted from over 3.5 thousand human faecal samples from sporadic cases of acute gastroenteritis referrals to seven regional Public Health Laboratories in England and Wales. An algorithm for PCR-based detection and identification was employed, which consisted of a screening PCR followed by species identification with PCR-ELISA.

Our study addresses the differential prevalence of enteropathogenic campylobacters, the efficacy of PCR compared with culture-based detection, and provides new data about non-*C. jejuni/C. coli* and mixed infections.

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## Detection And Characterisation Of New *Campylobacter* Species Using Molecular Methods: 'The Sequence Of Events'

CD20

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As part of a hygiene survey of abattoir workers, *Campylobacter*-like organisms were isolated from the faeces of healthy individuals. Two strains were identified as *Campylobacter* both by phenotype and by genus-specific PCR. They formed a unique DNA homology group within the genus and were phenotypically distinct from known *Campylobacter* species. Analysis of the nucleotide sequence of the 16S rRNA gene demonstrated that they constituted a new species, for which we propose the name *C. lanienae* sp. nov.

From a PCR screen of faecal samples from healthy individuals for *Campylobacter* we detected 16S rDNA genus amplicons to which we could not attribute a species. Three were sequenced and phylogenetic analysis showed that these were 99% similar, and formed a novel group, termed '*Candidatus* Campylobacter hominis'. From phylogenetic data we predicted the bacterium would be a fastidious anaerobe. Specific primers were designed to monitor the success of isolation. Using a combination of prolonged anaerobic incubation, membrane filtration and immunomagnetic separation, the organism was grown. The isolates 16S rDNA sequence was identical to '*Candidatus* C. hominis' sequences. Relative DNA-DNA homology, mol% G+C ratio, SDS-PAGE and phenotypic numerical taxonomy confirmed that *C. hominis* sp. nov. is a new *Campylobacter* species, that may be a commensal in the human gut.

**CD21** Enzyme Immunoassay (EIA) and Westernblot (WB) in *H. pylori* Diagnostic and the meaning of cagA-Diagnostic with WB and IFT

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The aim of this study was to compare i) the invasive *H. pylori* diagnostic methods [H.p.-Urea-test (HUR), culture and histology], ii) the results of serological EIA assay as screening to those obtained by Westernblot (WB) as confirmation regarding their validity by 206 patients with gastrointenstinal complaints and iii) to establish whether cagA-types among H.p. isolates are associated with disease. The cagA status of H.p. isolates (n= 38) were assessed by PCR and IFT. The sensitivity of the HUT, culture and histology was 84.9, 73.6 and 62.3 % respectively, that of a combination of HUT with culture or histology 96.2 %. Sensitivity and specificity for IgG-EIA was 80.4 and 78.6 %, for IgA-EIA 90.2 and 59.2 %, for IgG-WB 92.2 and 67.4 %, for IgA-WB 80.4 and 65.9 % respectively. The combined usage of IgA and IgG increased the sensitivity in WB and EIA. A higher sensitivity (98.0 %) was obtained by a combination of EIA plus WB. In the cagA-diagnostic the cagA-WB (patients sera) and the cagA-IFT (bacteria strains) were validated by means of PCR cagA determination. The sensitivity and specificity of the cagA-antibodies-WB and the cagA-protein-IFT was 100 and 76.5 % and 35.0 and 71.4 % respectively. A significantly higher prevalence of infection with the cagA-positive strains was found by chronic duodenitis, gastric and duodenal ulcus. In WB, no difference was detected by the clinical picture in relation to the cagA-distribution. **Conclusion:** In the invasive diagnostic a combination of HUT and culture (susceptibility test possible) is recommended. By questionable results in EIA is a further IgG-test in WB recommendable. The cagA-WB let no evidence pertaining to the pathogenicity of the H.p. infection.

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**RD1** **PREVALENCE, ANTIMICROBIAL SUSCEPTIBILITY PATTERNS AND CLINICAL SIGNIFICANCE OF *ARCOBACTER* IN STOOL SPECIMENS.**

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WHO Coll. Center for Enteric Campylobacter Sint-Peter[1], Brugmann[2] Univ. Hosp. Brussels, Univ. of Ghent[3], Belgium.

**Objectives:** A prospective study was carried out in our hospital to determine the prevalence of *Arcobacter* spp. in the population presenting with diarrhoea and the clinical picture associated with this pathogen.

**Methods:** From November 1995 to March 1999, all stool samples submitted to our laboratory were cultured for common enteropathogens and *Arcobacter* spp. (enriched broth was cultured on *Arcobacter* Specific Medium (ASM)). The plates were incubated for 3 days at 25°C. The antimicrobial susceptibility patterns were tested by an agar dilution method. All charts from patients with *Arcobacter* spp. infection were reviewed retrospectively.

**Results:** A total of 33360 stool specimens were collected from which only 53 *Arcobacter* strains were isolated from 41 different patients. Among the 53 strains, 49 were identified as *A. butzleri* and 4 as *A. cryaerophilus*. High resistance rates were found to amoxicillin/clavulanate (42%); chloramphenicol (49%); nalidixic acid (54%) and co-trimoxazole (100%). Most *Arcobacter* strains were sensitive to ciprofloxacin and erythromycine (95% and 92% respectively). The most common clinical features were acute watery diarrhoea and abdominal pain.

**Conclusion:** These results suggest that *Arcobacter* spp. may be potential enteropathogens but optimized methods for their isolation are required to assess their role in diarrheal disease. Fluoroquinolones or macrolides should preferably be used for the treatment of severe *Arcobacter* infections.

**NOTES**

RD2

**Arcobacter butzleri in hospitalized patients.**

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**Introduction:** *Arcobacter butzleri* has recently been described as a human intestinal pathogen,often causeing outbreaks of diarrhoeae in institutions and communities. **Materials & methods:** From April 1997 until June 1999 stools from about 4250 hospitalized patients were cultured for intestinal pathogens in our laboratory. The fecal specimens were diluted 1:1 in phosphate buffered saline pH 7.4. For detections of *Campylobacter* and related organisms the suspensions were filtered through a filter with a pore-size of 0.8 μm on 5% horse blood agar plates with yeast-extract. The filters were removed after 45 min aerobic incubation and the plates were incubated microaerobic at 37°C for 2 days. In addition, the fecal suspensions were grown microaerobic on selective *Ca mpylobacter* media at 42°C for 2 days. Archobacter butzleri was isolated and identified according to the probability matrix described by On et al. 1996. **Results & discussion:** Confirmed *Arcobacter butzleri* was isolated from three patients and suspected but not yet confirmed in a fourt patient. The age of the patients were 57 to 90 years (mean 70 years). According to patients record symptoms reported were diarrhea and abdominal pain. All patients were hospitalized for other diseases as cardial insufficiency, recent lung transplantation, dissiminated cancer and nephrotic syndrome. One patient died because of complicating pneumonia. All cases occurred sporadically. Thus, sporadic cases of *A. butzleri* infections may occur in elder patients at reference hospitals admitted for other diseases. Severe lethal complications may occur in these elderly patients.

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CE1

UREASE-POSITIVE THERMOPHILIC STRAINS OF CAMPYLOBACTER (UPTC) ISOLATED FROM SEAGULLS (LARUS SPP.)

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The possibility exists that environmental contamination by campylobacters might be mediated by wild birds. Among birds, gulls are thought to be responsible for serious deterioration of water quality in storage reservoirs and the transfer of campylobacters from gulls to man via water has been proposed. In paticular, C. lari has been isolated mainly from seagulls. Therefore, an attempt was made to isolate and analyze UPTC, demonstrated to be a variant of C. lari, from seagulls.

Three strains of UPTC (A1, A2 and A3) were identified by biochemical characterization after isolation from the faeces of seagulls on the coast around Belfast in N. Ireland in 1996. The biochemical characteristics of the 3 strains were identical to those of the strains described previously. Analysis by pulsed-field gel electrophoresis (PFGE) after digestion of genomic DNAs with ApaI and SmaI demonstrated that the respective PFGE profiles were indistinguishable. The PFGE analysis also suggested that the 3 genomes were approximately 1,810 kb in length. This is the first example of the isolation of UPTC from seagulls in Ireland Island.

Our previous discovery of the occurrence of UPTC in the Far East, in Japan, in conjunction with the cited reports of UPTC in Europe and the present study strongly suggest a worldwide distribution of UPTC. Thus, further investigations of UPTC are clearly warranted from a public-health perspective.

CE2 PREVALENCE OF CAMPYLOBACTER JEJUNI AND UREASE-POSITIVE THERMOPHILLIC CAMPYLOBACTER(UPTC) IN CROWS

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Campylobacter jejuni and UPTC is considered an important etiologic agent of anthropozoonosis, and is most frequently isolated as a causative agent of sporadic diarrhea in humans. In this study, we attempted to isolate these bacteria from crows, which are closely involved in the living environment of humans, and investigated the isolates by molecular epidemiology. From 255 crows captured during extermination of harmful birds, isolation of both bacteria was attempted.

As a result, Campylobacter jejuni was isolated from 9 crows(3.5%). The nine isolates identified as C. jejuni were eight serotypes($Z_s$, F, K, Y, U, L and $Z_T$). Regarding UPTC, bacteria were isolated from two crows (0.8%). These were the initial isolation in Japan, demonstrating the presence of this bacteria in Japan.

DNA of each isolate was molecular epidemiologically investigated by pulse-field electrophoresis. Nine isolates of C. jejuni were classified into 10 types. Among three places where the materials were collected, a single type was isolated from two places, but 8 types were isolated from the remaining one place. Regarding UPTC, the two isolates from the same district were identical types.

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CE3 Restriction fragment length polymorphism (RFLP) analysis using random chromosomal gene probes for epidemiological analysis of *Campylobacter jejuni* infections

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We have evaluated the ability of a new genotyping method for *Campylobacter jejuni*, based on restriction fragment length polymorphisms (RFLPs) using random chromosomal gene probes. Two unrelated cosmid clones P14 and P15 (respectively containing 30 and 35 kb genomic DNA fragments of *C. jejuni* strain OH4384) were used as probes. DNA of *C. jejuni* strains digested with each of three restriction enzymes, *Hha*I, *Hae*III, and *Hpa*II, was analyzed by Southern hybridization using each of the probes. RFLPs detected by this method were stable for isolates from a patient with long-term infection, for strains from patients within a family, after passage on agar plates, and in vivo. In addition, this technique distinguished accurately between outbreak and sporadic strains arising in the same geographic area in the same time period Thus, the method reported provides a stable and discriminating means for identifying *C. jejuni* strains, and should be useful for epidemiological analyses.

Pre-harvest surveillance of *Campylobacter* and *Salmonella* in broilers in Denmark. CE4

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Two national surveillance programmes in broiler flocks are carried out in Denmark. Since January 1998 for *Campylobacter* and since 1989 for *Salmonella*.
In 1998 57,000 samples for *Campylobacter* representing 5,700 broiler flocks were taken at ten different abattoirs, and 22,055 samples for *Salmonella* were taken from the same flocks in the broiler houses at the farms.
The samples for *Campylobacter* were taken as swabs from the cloaca, ten swabs from each batch of broiler flocks. The samples for *Salmonella* were taken as "socks" by pulling 15 cm long cotton tubes over the footwear and turning the socks when walking around in the house so that all parts were exposed. Five pairs of "socks" were taken from each flock.
47.0% of the swabs were *Campylobacter* positive. Most positive samples were found during August and September, while the lowest number of positive samples were found during January and February. 7.0% af the flocks were *Salmonella* positive.
Among the *Campylobacter* positive samples *C. jejuni* was cultured in 85% of the cases, *C. coli* was cultured in 11% of the cases, the remaining species were mainly *C. lari, C. hyointestinalis* and *C. upsaliensis*.
316 producers participated in the study, each having from 1 to 16 different houses at their property, in each house up to 8 different flocks (rotations) were raised during the study period, each flock was slaughtered in up to 6 batches.
For each flock presence of *Campylobacter* and *Salmonella* was recorded in order to estimate the number of flocks which were negative to both infections, the number of flocks which were positive to both infections and the number of flocks which were positive to only one of the infections.
In conclusion no correlation between occurrence of *Campylobacter* and *Salmonella* infections was found in Danish broilers.

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Epidemiological and Clinical Patterns of *Campylobacter* Infection at Red Cross Children's Hospital During 1998. CE5

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Epidemiological and clinical features of infection with *Campylobacter* were examined in paediatric diarrheal patients in an attempt to further define the characteristics of disease. Data of 332 culture-confirmed patients with *Campylobacter* enteritis attending Red Cross Children's Hospital were collected over one year period from January 1 to December 31, 1998. Gender was equally distributed and the ages ranged from less than one month to 71 months of age, with a mean of 18 months. A total of 403 isolates were collected from the 332 patients. *C. concisus* with its prevalence of 33%, was the commonest species isolated. The next most frequent species/subsp. were *C. jejuni* biotype 1 (22%), followed by *C. upsaliensis* (21%), *H. fennelliae* (8%), *C. jejuni* subsp. *doylei* (7%) and six other species (9%). The number of isolates during the 3-month period towards the end of South African summer was notably higher than during the corresponding 3-month winter period (45% vs. 14%, $p < 0.01$). Seasonal variations in species distribution were also observed. Clinical characteristics such as the severity of diarrheal symptoms and the consistency of the stool (watery vs. loose) were similar in the species identified. Age-specific infection by species was observed.

CE6 Thermophilic *Campylobacter* spp. in sheep. Risk for human infection? NOTES

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As a source of infection for human campylobacteriosis, other food animals than poultry have lately gained interest. In a nationwide survey in Sweden, sheep were sampled at slaughter and faecal samples were examined for thermophilic *Campylobacter* spp. Samples were taken at 7 different slaughter-houses, from 583 ewes and 404 lambs from September to November in 1998. Selective media were used for culture and samples were incubated at 42° C in microaerophilic atmosphere. From colonies with typical appearance, material was examined for motility by microscopy, and tested by pheno- and genotypic methods. Based on traditional phenotyping, an average of 10% of the samples contained campylobacters. The recovery rate was higher in samples from lambs (13%) than from ewes (7%). PCR was performed with primers specific for the thermophilic *C. jejuni*, *C. coli*, *C. lari* and *C. upsaliensis*, followed by restriction enzyme analysis for differentiation between the four *Campylobacter* species. This genotyping showed that the isolates, phenotypically characterized as *Campylobacter* spp consisted of approximately 60% *C. jejuni*, 10% *C. coli* and 30% non-thermophilic (PCR-negative) *Campylobacter* spp. The latter isolates were further tested for growth at lower temperatures and in aerobic atmosphere. The results indicate that about 20% of the isolates were in fact not *Campylobacter* but *Arcobacter* spp.
These data show that the prevalence of thermophilic *Campylobacter* spp. in sheep at slaughter is less than 10%. However, the finding that the majority of isolates were *C. jejuni*, which is the most common cause of human campylobacteriosis, is noteworthy. Whether the isolated non-thermophilic campylobacters and related organisms pose a risk for public health needs to be clarified.

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CE7 **The annual incidence and seasonal distribution of a *Campylobacter jejuni* strain associated with human infections. A proposal for a bovine reservoir and potential sources of transmission.**

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This study defines a *Campylobacter jejuni* strain (Penner serotype HS4 'complex': Preston phage-group 55) which has been frequently associated with human gastro-enteritis in the UK over a seven year period. This strain shows a seasonal distribution which coincides with the seasonal peak incidence of Campylobacter infections in the UK. The organism appears to have a global distribution and has been isolated in cattle from geographically diverse sources and has been demonstrated in samples from the UK, Canada, Australia and New Zealand. The strain has been demonstrated to have been the causative agent of four milkborne outbreaks in the UK over a period of 15 years and is frequently found in bovine offal samples. This study defines the strain and describes the proportion of human disease attributable to the organism and its seasonal incidence. We develop the hypothesis to suggest a bovine reservoir for this organism and identify potential vehicles of infection for this strain.

## Epidemiological study of *Campylobacter* clinical isolates in the West of Ireland. CE8

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Epidemiology of *Campylobacter* infections in the West of Ireland were investigated during the latter half of 1996. 100 clinical isolates were collected at random during this period. Strains were characterised by various methods: biotyping, antibiotic profiles, plasmid profiling, ribotyping and flagellin gene analysis.

69% of isolates were identified as *C. jejuni* Biotype I and 27% were identified as *C. jejuni* Biotype II. The remaining isolates were atypical. Antibiotic profiles were carried out on all isolates using a wide range of antibiotics. 20 of the isolates were resistant to various antibiotics with some resistant to more than one antibiotic.

Ribotyping analysis was carried out using both 16S and 23S rRNA gene probes. DNA from all isolates was restricted separately with *Hind* III and *Hae* III. Following ribotype analysis it was found that DNA restricted with *Hind* III and probed with 16S/23S probe produced 12 ribopatterns while DNA restricted with *Hae* III produced 18 ribopatterns.

Flagellin gene analysis was carried out by the amplification of the *fla* A and B genes by PCR. Following digestion of the PCR product, fragments were separated by electrophoresis on 6% acrylamide gels. Analysis of the restriction fragment length polymorphisms (RFLP) showed that 3-24 bands were present for each strain.

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## Theoretical consequences of recombinations, gene conversion, and mutations in the flagellin gene locus on *fla* typing systems. CE9

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Genetic typing methods are now generally applied on *Campylobacter* ssp. for subtyping purposes. The RFLP analysis of PCR fragments derived from the flagellin genes (*fla* typing) is a sensitive, simple and fast method, that is presently available in a number of varieties. Reports on assumed instability of the flagellin gene locus enabled us to predict how sensitive these *fla* typing methods are to genetic changes within a bacterial lineage. The changes that occur are point mutations, recombinations or gene conversion between the two *fla* genes of one locus, and recombinations between *fla* loci obtained by horizontal gene transfer. The differences between the *fla* typing methods used at present are choice of *fla* gene (*flaA*, *flaA*+B, and separate *flaA* and *flaB*-specific PCR reactions), primer choice (composition and location), and restriction enzymes used. The major difference in sensitivity to proposed genetic changes are expected in *flaA*-typing versus *flaA+B* typing. Sequence comparison identified regions of high and low variability. Regions at both ends of the *fla* genes appear to be momophyletic, thus recombination does not significantly contribute to diversity in these regions. The central region has a unique phylogeny. PCR-RFLP bands that conrrespond to the conserved portions of the *fla* gene will be more commonly present and are more likely to be linked to specific sub-populations of *Campylobacter*. The consequences of these theoretical considerations to the applications of *fla* typing are discussed..

**CE10** Effect of the sanitary barriers on the colonization of free broilers flocks by *Campylobacter* spp.

**NOTES**

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In order to evaluate the effect of sanitary barriers on the colonization by *Campylobacter* in a special french broiler system, four farms were investigated. The particularities of this breeding are a long rearing period (minimum 81 days), good quality of food (minimum 70% of cereals) and access to an open area after 6 weeks of breeding.
For this study, some advises had to be respected: obligatory change of farmers'boots, two areas in the entry room (one dirty for their outside boots and one clean for their inside boots), no domestic animals in the poultry farm and in the open area, and chlorinated drinking water for chicken.
Samples (outside soil, fresh dropping birds, environmental swabs, food and water) were collected at three different times: the day of arrival of the chicks, just before the exit in the open area and just before the departure of the animals to the slaughterhouse.
Until the exit of the chickens in the open area, all the samples collected inside the build (faeces, swabs, food and water) were *Campylobacter* negative. Some soil samples and all the chicken faeces samples done before slaughtering were *Campylobacter* positive.
The *Campylobacter* isolates were characterized by macrorestriction using two enzymes (*Sma*I and *Kpn*I). In two farms, some strains isolated from either the soil or the faeces samples showed identical patterns.
This study allows us to conclude that sanitary barriers are effective to keep poultry *Campylobacter* free, and that the soil can be a source of contamination of the chickens by *Campylobacter* spp.

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**CE11** A multiplex PCR for silmutaneous identification of *Campylobacter jejuni* and *Campylobacter coli.*

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Discrimination between the closely related species *C.jejuni* and *C.coli* is based only on the hippurate hydrolysis test. This phenotypic distinction is not always accurate. Consequently identification of the two species based only on this single test is unreliable. The Polymerase Chain Reaction (PCR) looks to be a good and rapid genetic assay to identify *C.jejuni* and *C.coli*. We have developed a multiplex PCR which allows the detection of the bacteria and the identification of the two species in one tube PCR.
Three pairs of primers were selected from three genes, *16sRNA*, *mapA* and *ceuE* genes, for firstly the detection of the genus *Campylobacter*, secondly the identification of the *jejuni* species and thirdly, the identification of the *coli* species, respectively. The amplification generates three DNA fragments when the DNA of the two species are present in the PCR tube; one with a length of 857 bp for the genus *Campylobacter*, a second with a length of 589 bp for the species *jejuni* and a third fragment with a length of 462 bp for the species *coli*. For the development of the multiplex PCR, two conditions were necessary : (i) a close melting temperature of the primers for using the same annealing temperature and (ii) three PCR products with different sizes for vizualisation of the three DNA fragments on agarose gel after electrophoresis.

**Assessment of the *in vitro* and *in vivo* stability of epidemiologic markers for typing *Campylobacter jejuni* evaluated by serotyping, PFGE and RiboPrinting** CE12

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[1]Statens Serum Institut, Copenhagen S, Denmark; and [2]Danish Veterinary Laboratory, Copenhagen V, Denmark

Validation of a typing method includes evaluation of its performance. Several performance criteria are essential, in particular stability, typeability, reproducibility and discriminatory power. The stability of epidemiologic markers condition the ability of a typing system to recognize the clonal relatedness of strains derived from a common ancestor*. To determine the stability of conventional serotyping (Penner) as well as some better discriminating molecular typing methods, we investigated the effect of genetic events on serotypes, PFGE- and RiboPrinting DNA profiles of *C. jejuni* in an *in vitro* and *in vivo* model. Three isolates of different serotypes and antibiograms were subcultured up to 50 times over a 4-month-period and the same isolates were intragastrically administered to mice separately and as a mixture of two different strains to allow intergenetic exchanges during colonisation. Fecal samples were cultured at regular intervals up to the end of the experiment at day 26. After 50 subcultures and 26 days of colonisation, all isolates showed stable serotypes and no significant alterations in PFGE- and RiboPrinting DNA profiles were seen.

In conclusion, serotyping as well as the DNA-based typing methods PFGE and RiboPrinting were shown to be stable methods for typing *Campylobacter jejuni*.

*M. J. Struelens and ESGEM 1996. Clin. Microbiol. Infect. **2**:2-11.

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Comparison of serotype distribution in campylobacter isolates from human infections in England, Wales and Belgium. CE13

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Campylobacter is the most commonly reported bacterial agent of gastro-intestinal disease in North West Europe yet, by comparison with salmonella, few isolates are typed beyond species level and detailed studies of campylobacter epidemiology across Europe are not therefore possible.

Campylobacter isolates from human infection in North West England and Wales are routinely referred for typing to the Public Health Laboratory Service Reference Unit. During 1998 7,693 *C. jejuni* and 593 *C. coli* were typed using the LEP serotyping method supplemented by phage-typing for sub-division within the most common serotypes. A sample of 78 *C. jejuni* and 20 *C. coli* isolates from human infection in Belgium, was typed using the same methods in order to compare the relative incidence of the common types in the two countries.

The 12 predominant *C. jejuni* serotypes were common to all three populations but their rank order differed. In *C. coli* over 50% of isolates from each country belonged to serotypes HS56 or HS28 but again the proportions varied between samples.

CE14 **An increased prevalence of *Campylobacter jejuni* HS 19 in human campylobacteriosis in the United Kingdom**

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Between November 1997 and April 1999, CRU identified 136 cases of enteritis associated with *C.jejuni* HS19. This type had not previously been observed among clinical isolates referred to the Unit during the previous 18 months. The frequency of occurrence of HS19 isolations exhibited seasonal variation, with a notable bi-modal peak occurring between May and September followed by a lesser peak in January 1999, mainly attributable to an outbreak involving ≥10 cases in a school community.

Further subtyping of the HS19 strains by phage type showed 86% to be PT 2. Pulsed-field gel electrophoretic profiling (PFGE) using the restriction enzymes *Sma1* and *Kpn1* identified a predominant epidemic type (PT 2; S1/K1) which comprised 51% of all isolates, including those associated with the school outbreak. The remaining isolates were defined by a combination of 5 phage types, 15 *Sma1* and 5 *Kpn1* profile types.

Infection with *C.jejuni* HS19 has been implicated as a predisposing factor in the development of Guillain-Barré Syndrome (GBS). Three of the 136 patients (2.2%) infected with HS19 strains are known to have developed GBS as a sequelae to campylobacteriosis, and in one of these the causative strain was type PT 2; S1/K1.

The detailed strain characterisation undertaken in this study has identified the emergence of a well-defined clone of *C.jejuni* HS19 that has become widely distributed in England and Wales.

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CE15 Heat stable antigens of *Campylobacter jejuni* and their detection by direct agglutination and passive haemagglutination.

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Variations in *C. jejuni* heat stable antigens may be detected by passive haemagglutination, as in the typing method of detection Penner & Hennessey, or by direct whole cell agglutination using absorbed antisera as described by Frost, Oza et al. These differences in method, coupled with the use of absorbed antisera in the direct agglutination method, results in incomplete concordance between them, although the same type strains have been used to raise the antisera in both schemes. Indeed, for some serotypes, antisera pre-incubated with homologous sensitised red blood cells still exhibit specific reactions in the direct agglutination test indicating a difference in the antigens/epitopes detected. These differences can be used to explore the nature of the antigen detected.

In this study 450 unselected clinical isolates have been typed by both methods. Among the more prevalent serotypes, a high degree of concordance was seen with serotypes HS6, HS19 and HS11. The majority of isolates assigned to the Penner HS4 complex [cross-reacting serotypes HS4, 13, 16, 50 and 65] typed as HS50 using the LEP method. Among the remainder HS13 and HS16 could be distinguished as discrete types. Relationships between HS1, HS2 and HS44 are more complex with isolates clustering differently depending on the agglutination

## Automated ribotyping of *Campylobacters* from poultry

CE16

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We started to type large numbers of *Campylobacter* isolates from ten broiler production farms in the framework of a comprehensive *Campylobacter* monitoring project (see also Jacobs-Reitsma, *et al.*: Collaborative research project on *Campylobacter* epidemiology and control in poultry production). The project aims at elucidation of epidemiological factors in *Campylobacter* colonization of broiler flocks and at evaluation of specific intervention procedures to prevent or reduce colonization in these flocks. The RiboPrinter® Microbial Characterization System (Qualicon™) was selected to provide characterization and identification with high throughput, reproducibility and both automated experimentation and data analysis. Automated ribotyping with *Pst* I as restriction enzyme identified 29 different types (RiboGroups) in a set of 48 reference strains isolated from geographically "dispersed" poultry flocks (discriminatory power D = 0.97; Hunter, 1990, JCM 28:1903-1905). On basis of these dynamic RiboGroups characterized, static patterns were created and used for automated custom identification and counting ("numerical catalogue-ing") of *Campylobacter* ribotypes in about 3000 isolates, mainly obtained in the monitoring project since September 1997. This led to the definition of an additional 60 ribotypes. Clustering of these custom identification profiles reflects speciation results from our *C. jejuni/coli* identification PCR.

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## Collaborative research project on *Campylobacter* epidemiology and control in poultry production

CE17

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Campylobacteriosis in man is mainly a food-borne infection, in which poultry products, play an important role. A comprehensive Campylobacter project on ten broiler farms was initiated in September 1997. Sampling at regular intervals during 10 consecutive production cycles included 784 broiler flock samplings of 10 fresh caecal samples each, as well as 308 environmental samplings from other farm animals, boots, water, darkling beetles, mice, etc. The Riboprinter® Microbial Characterisation System (Qualicon™) was used for genotyping of Campylobacter isolates. Detailed information on farm management and zootechnical data were recorded for each flock.
At slaughter, Campylobacter was isolated from 177 of 329 (54%) flocks. Surprisingly, Campylobacter was already isolated from 17% of the 197 flocks sampled at 2 weeks of age. The percentage of contaminated flocks per farm varied between 30% to 89%. This variation, however, could not be attributed to particular epidemiological factors. Only 26 environmental samples yielded Campylobacter, originating from pigs (20), dogs (2), aviary birds (2), pigeons (1) and cattle (1). Pigeon and cattle isolates represented a different ribotype than poultry isolates from the same farm. However, pig isolates (partly) matched with the poultry isolates on the 3 farms involved. Sixteen parent flocks on five separate farms, regularly delivering progeny to seven of the farms under study, were sampled on one occasion and all 16 flocks were found to be colonised with Campylobacter. Ribotyping of these parent flock isolates is carried out at the moment.

**CE18** **AFLP-Genotyping of *Campylobacter fetus***

**NOTES**

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*Campylobacter fetus* is divided into two subspecies fetus and venerealis. The high homology at genetic level is in contrast with significant differences in clinical presentation. Subspecies venerealis is the cause of bovine venereal campylobacteriosis. It is genitally transmitted inducing abortion and/or infertility. Subspecies fetus can cause abortion in sheep after oral infection. This subspecies is not restricted to sheep but is present in many animal species. In contrast with subsp. venerealis, subsp. fetus can not be eradicated due to it's common occurrence in the environment. Subspeciation is important for both clinical and economic reasons. Statutory import requirements for semen and embryos for some countries specify freedom either from both subspecies or from subsp. venerealis only. However phenotypic subtyping of *C. fetus* is difficult. Differentiation is based on growth in presence of 1% glycine or 0.1% sodium selenite, and these subtyping-methods can give ambiguous results. Recently AFLP has been successfully applied to the molecular typing of Campylobacters. Therefore AFLP was performed on 71 *C. fetus* clinical isolates from different geographical locations (Africa, several EC-countries) and hosts. As expected, the genetic relationships between the different isolates were high. However, in general, patterns differentiating the subspecies were observed. Thus AFLP-subtyping seems to be a suitable method for subspeciation. Nevertheless some strains showed anomalous patterns, inconsistant with phenotyping and PCR-based genotyping results. Whether these results reflect different genetic lineages is now under investigation. The value of phenotyping, PCR-genotyping and AFLP for subspecies differentiation will be reported.

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**CE19** **Comparative analysis of four genotyping techniques for the thermotolerant Campylobacter species *C. jejuni* and *C. coli.***

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For epidemiological tracing of the thermotolerant Campylobacter species *C. jejuni* and *C. coli* good typing techniques are a necessity. The resolution of the traditionally used phenotyping techniques such as serotyping proved inadequate. Genotyping techniques were therefore developed, of which flagellin typing (*fla* PCR RFLP), Pulsed Field Gel Electrophoresis (PFGE) and in lesser extent Ribotyping are now the most commonly used techniques. Recently, the Amplified Fragment Length Polymorphism (AFLP) fingerprinting technique has been adapted for Campylobacter typing. This study was conducted to compare these four genotyping techniques, and to investigate their use for epidemiological typing of Campylobacter. A set of fifty Campylobacter poultry isolates from The Netherlands (46 isolates) and neighboring countries (4 isolates) was used for this comparison. AFLP and PFGE were the most discriminatory, both identifying 38 different types. Flagellin typing discriminated 34 different types whereas Ribotyping discriminated 26 different types. In some cases combining the results of AFLP, PFGE and flagellin typing led to distinction between isolates that appeared genetically identical by one method. Therefore we recommend the use of multiple methods for epidemiological typing of Campylobacter. Data will be presented arguing the advantages and disadvantages of all methods. We conclude that the use of AFLP is preferred, either as single method but preferably in combination with other methods.

CE20

Sequential spread of *Campylobacter* infection through a multi-pen broiler house

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Birds within a commercial broiler house composed of 72 separate pens were monitored weekly for Campylobacter infection from 18 days of age until slaughter. The pens were contained in 4 main blocks, each with 18 pens separated by wire mesh. Each pen held 100 birds. Cloacal swabs were collected from 5 birds in each of 12 pens identified by random selection for regular sampling. One colony per bird was speciated and further sub-typed by PCR/RFLP of the *fla* A and *fla* B genes. Campylobacter infection was first detected in birds aged 32 days, in two pens only, at the rear of the broiler house. One week later, all birds sampled from 12 pens were positive for campylobacters. All strains isolated up to this time were identified as *C. jejuni* and were the same genotype (*fla* type 1.9). Subsequently, at 46 days of age, some birds in 5 of the pens towards the rear of the house were shown to be infected with a new genotype (*fla* type 3.7) whilst the majority of birds remained infected with the original infecting strain. The genetic difference of the two infecting strains was further confirmed by pulsed field gel electrophoresis (PFGE) using *Sma*I endonuclease. Normal biosecurity procedures were in place during the investigation including the employment of boot-dipping at the main entrance to the house. The initial appearance of both infecting strains in birds housed in pens at the rear of the house, indicating a common point of entry, was unexpected. However, it was subsequently established that a door at the back of the house had been used for removing dead birds. The experiment has now been repeated with the rear door closed off and these results will be communicated. The multi-pen broiler house has provided a useful model for studying sequential spread of *Campylobacter* infection within the broiler house which, combined with a suitable sub-typing scheme for isolates, can facilitate the identification of point(s) of entry of infection into the house.

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CE21

A Network for the Standardisation and Harmonisation of Campylobacter Molecular Typing Methods (CAMPYNET).

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A network was established on 1st September 1998 to harmonise and standardise molecular typing techniques for *C.jejuni/coli*. The network is funded by the EC for 3 years and formally comprises 24 participants from 11 countries. The project is planned in two phases. In phase 1 a reference strain set will be established and standard operating procedures for *fla*-typing, PFGE, AFLP and data handling will be recommended. In phase 2 the technologies will be transferred to all the participant laboratories. Six working groups have been established to inplement this plan - WG1 will collect, characterise, select and distribute the reference strain set; WG2, WG3 and WG4 will produce the recommended procedures for *fla*-typing, PFGE and AFLP respectively; WG5 will evaluate currently available software for the handling of data from the various typing technqiues and will establish communications within the network and WG6 will investigate any alternative and novel typing technologies. To date the reference strain set collection is near completion. This strain set will be made available, at cost, to non-participant laboratories on request in the future. Comparisons of various typing procedures are ongoing in several laboratories and a website has been established (www.svs.dk/campynet/). Further progress will be reported.

**CE22** The molecular epidemiology of Campylobacters isolated from broiler flocks and their environment J.E.SHREEVE[1], M.W.MARRIOTT[2], M.TOSZEGHY[1], T.J.HUMPHREY[2] AND D.G.NEWELL[1] 1)Veterinary Laboratories Agency (Weybridge) UK. 2) PHLS Exeter, UK

The sources and routes of transmission of *Campylobacter* infection in broiler houses is largely unknown. A molecular sub-typing method (PCR/RFLP of the *fla*A and *fla*B genes ) has been applied to differentiate *C. jejuni/coli* strains isolated during regular sampling of four successive crops of birds and from samples taken from both the internal and external environments of a commercial broiler house. Internal samples included drinkers, litter, feed-lines, walls, corners, posts and fans. External samples included soil, feaces, foot-swabs, puddles, ditches, boots and wild bird faeces. The results have shown that a wide diversity of *Campylobacter* genotypes exist in the environment around the broiler house and can act as potential sources of infection within houses. Strains colonising the 1st and 3rd flocks were the same *fla* genotype as detected earlier from cow faeces indicating a likely source of infection in these cases. A different genotype was identified amongst strains colonising the 4th flock which was also recovered from the soles of workers boots after boot-dipping suggesting a route of transmission into the broiler house. Results from these studies indicate that infected birds can induce heavy contamination of the internal broiler house environment. However campylobacters were not recovered from inside the house following cleansing and disinfection suggesting that carry-over from one crop to the next by this source is an infrequent event. Moreover whilst contaminated feed-lines and drinking water can aid the spread of infection within the house there is no evidence that these are the sources of campylobacters. In ours studies whilst infected birds can become colonised by further strains it would appear to be comparatively rare for one bird to have more than one genotype present.

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**CE23** The comparison of seasonality in campylobacter infections in humans and chickens from three European countries. D.G. NEWELL[1], E.HARTNETT[1], M. MADSEN[2], J. ENGBERG[3], T. HALD[4], A. WEDDERKOPP[2], A. ENGVALL[5]. 1) Veterinary Laboratories Agency (Weybridge), UK. 2) DVL, Aarhus, DK. 3) Danish Zoonosis Centre, DVL, Copenhagen, DK. 4) State Serum Institute, Copenhagen, DK. 5) SVS, Uppsala, SE

From national surveys seasonality in cases of human campylobacteriosis in industrialised countries has been recognised for many years. The reasons for this are unknown but a concurrent seasonality in major sources such as poultry meat have been suspected. Until recently national or even large surveys of poultry colonisation with campylobacters have not been available for comparison. However several European countries, including Denmark, Sweden and the UK, now have suitable data for both human and poultry flock campylobacter infections over a period exceeding one year. In the UK numbers of human infections are recorded weekly. This data clearly demonstrates two peaks, the largest in week 25 with a smaller one in week 36. There is no national survey of poultry for campylobacter but recently 2 major national poultry companies have surveyed all their flocks for periods of up to one year. Both poultry surveys indicate muliple peaks in colonisation. Unfortunately methods of data collection make direct comparison of human and flock infections difficult. Nevertheless, although an early summer peak in flock infection generally coincides with the largest human peak other peaks are clearly unconnected. Moreover when storage and retailing times are taken into account the association between the summer peaks may become even weaker. Similar disparities between peaks in human and poultry flock infections observed in national surveys in Sweden and Denmark will also be presented. The general conclusion of this study is that at least some of the seasonality in human infections cannot be accounted for by consumption of increased levels of contaminated poultry products. Alternative sources of human infection at these peak times need to be sought.

CE24

**Evaluation of methods for subtyping of *Campylobacter jejuni* during a recent outbreak in Kansas, USA.** Collette Fitzgerald[1], L.O. Helsel[1], M.A. Nicholson[1], S. J. Olsen[1], R. Flahart[2], P. I. Fields[1] [1]Centers for Disease Control and Prevention, Atlanta, GA; [2]Kansas Department of Health and Environment, Topeka, KS

In October 1998, CDC assisted in an outbreak investigation of campylobacteriosis at a school in Salina, Kansas. We received 22 *Campylobacter* isolates from Kansas: 9 outbreak isolates and 13 sporadic isolates. We tested the usefulness of various subtyping techniques in terms of typability and ability to distinguish outbreak strains from unrelated isolates. The methods employed were somatic **O** serotyping, PCR-RFLP analysis of *fla*A, DNA sequence analysis of 585 base pairs (bp) of *fla*A that included the short variable region, sequencing of the entire *fla*A gene, and pulsed-field gel electrophoresis (PFGE) using *Sma*I and *Sal*I. PFGE was the most discriminatory technique; 11 *Sma*I and 10 *Sal*I restriction profiles were observed. All outbreak strains were indistinguishable by PFGE, and the outbreak profile was not seen in the sporadic isolates. Seven of the 9 outbreak strains were serotype **O**:19, two were nontypable. *flaA* typing via PCR-RFLP grouped one sporadic isolate with the outbreak strains. Analysis of the DNA sequence of a 585-bp segment of *flaA* produced strain groupings similar to that generated by PCR-RFLP but further differentiated two *flaA* PCR-RFLP types (1 bp difference in the 585-bp region). The 585-bp sequence differentiated the one sporadic strain that grouped with the outbreak strains by *flaA* PCR-RFLP. Two sporadic strains were distinct by *fla*APCR-RFLP but differed only by a single base substitution in the 585-bp region. The entire *fla*A gene was sequenced from strains differing by a single bp in the 585-bp region. The two sets of strains that had identical *Dde*I restriction patterns and very similar 585-bp regions were very similar throughout *fla*A. The two strains that were distinct by *fla*A PCR-RFLP but very similar in the 585-bp region were 99.9% identical at the 5' half of the gene but more divergent at the 3' half of the gene (94% identity). PFGE was superior to all other typing methods tested for strain discrimination and was crucial for understanding the Kansas outbreak.

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CE25

**Use of high resolution subtyping to investigate the clonal relationships between *Campylobacter jejuni* serotype O:19 strains.** Collette Fitzgerald, M. Nicholson, L.O. Helsel, I. Nachamkin and P.I. Fields; Centers for Disease Control and Prevention, Atlanta, GA

Strains of *Campylobacter jejuni* can vary considerably in genotype between serotypes and within a single serotype. Recent data, looking at polymorphisms within the flagellin (*fla*A) gene and RAPD PCR suggest that *Campylobacter jejuni* serotype **O**:19 may represent a highly clonal population. We applied additional high resolution subtyping techniques to a diverse collection of *C. jejuni* **O**:19 strains to clarify these observations. Twenty-five **O**:19 strains were selected from the *Campylobacter* culture collection at the *Campylobacter* Reference laboratory at CDC and comprised strains isolated from different states in the USA between 1989 and 1999. Five isolates were from patients with GBS, 17 isolates were from sporadic cases of campylobacteriosis not associated with GBS, including 2 sets of 3 isolates associated with 2 different outbreaks and 3 isolates from primates. They were analyzed by PCR-RFLP of the flagellin (*fla*A) gene, pulsed-field gel electrophoresis (PFGE) with 2 different enzymes; *Sma*I and *Sal*I, and amplified fragment length polymorphism (AFLP) fingerprinting. A high degree of genomic similarity between strains was demonstrated. Restriction analysis of the *fla*A amplicons by *Dde*I gave 4 different *Dde*I digest profiles, but the majority of strains (19/25) had the same RFLP pattern. The choice of restriction enzyme dictated the discriminatory potential of PFGE. Analysis of *Sal*I digested genomic DNA produced 4 different restriction profiles with 20/25 strains having an identical profile, including the 2 sets of outbreak strains. *Sma*I was more discriminatory with 6 restriction profiles seen; however, they were similar, with only minor differences between banding patterns. In contrast, PFGE patterns of non **O**:19 isolates differed by up to 6 bands. AFLP fingerprinting is under way to assess the effectiveness of this technique for discriminating such a highly clonal group of strains.

**CE26** Infections with different Campylobacter species and serotypes in relation to clinical symptoms and medication

**NOTES**

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Clinical fecal isolates from 435 cases of *Campylobacter* enteritis enrolled in a case control study conducted in Denmark May 1996 to May 1997, were speciated and serotyped according to Penner serotyping. Information from cases on clinical symptoms and medication were evaluated in relation to species and serotype. Ninety-five percent of the isolates were identified as C. *jejuni* and 5% as C. *coli*. Among C. *jejuni* 28 different serotypes were identified. Serotype O:1,44, O:2, O:3, O:4-complex, O:6,7 and O:11 predominated and accounted for 74% of the isolates. Each of the other serotypes accounted for less than 3%. Of eight reported symptoms: nausea, abdominal pain, diarrhea, blood in stool, fever, headache, pain in joints and vomiting only the latter was more frequently seen among C. *jejuni* than C. *coli* cases. There was a tendency (but statistically insignificant) that infections with C. *jejuni* O:6,7 and C. *jejuni* O:11 were associated with fewer symptoms compared to the other serotypes. No differences were observed in receiving medication for the Campylobacter infection nor in the use of medication prior to illness onset between cases with different *Campylobacter* species or different serotypes of *C. jejuni.*

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**CE27** Interlaboratory Comparison of Automated Ribotyping of *Campylobacter* using the RiboPrinter® System

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A major challenge in carrying out large-scale epidemiological typing studies is the difficulty of finding high throughput methods that are sufficiently reproducible, discriminatory, and standardized to allow multiple reporting sites to generate and share typing data without having to share the bacterial isolates. The RiboPrinter® Microbial Characterization System (Qualicon, Inc.), an automated ribotyping instrument, has the automation to process high volumes of samples in support of large-scale typing studies. This evaluation assessed the ability of the RiboPrinter® system to provide the discrimination and reproducibility needed for such studies. The two cooperating laboratories each ran the same set of 124 blind-coded *Campylobacter* isolates. The data set consisted of 104 unique isolates from diverse sources. The additional 20 isolates consisted of duplicates of a randomly selected subset of the 104 original isolates. The isolates were independently ribotyped (one replicate) with the restriction enzyme *Pst*I on the RiboPrinter® system at each site and then the two data sets were merged electronically and further characterized using a standard visual refinement method. The final merged data set contained 59 unique clusters (RiboGroups). The largest RiboGroup consisted of 13 unique isolates. Of the 124 samples, 120 grouped correctly with their labeled replicate from the other site. Of the 20 duplicated samples, 16 showed all of the potential replicates falling into the same group. This excellent grouping performance, coupled with the automated instrument's capability to type these 124 isolates in less than one week, makes the RiboPrinter® system an attractive choice for the primary typing method for large epidemiological typing studies.

## Decline in campylobacteriosis in FoodNet sites

CE28

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*Campylobacter* infection continues to be the most commonly diagnosed bacterial fooborne illness in the United States. FoodNet was established to determine and monitor more precisely the burden of foodborne diseases including illness due to campylobacteriosis. FoodNet began population-based active surveillance for laboratory-confirmed cases of *Campylobacter* infections in 1996 in CA, CT, GA, MN, and OR, and added MD and NY in 1998 (total population 20.5 million). Collaborators in FoodNet sites contact each of 310 clinical laboratories at least once a month to assure reporting of cases. From 1996 to1998, *Campylobacter* was the most commonly isolated pathogen in FoodNet sites with an average incidence of 23 cases per 100,000 population per year. Thirty-seven percent of cases occurred during June-August. One hundred (1%) isolates were from blood. Among the 11,367 persons, 1154 (10%)were hospitalized and seven (0.1%) died. The average incidence varied by site from 10/100,000 in MD to 48/100,000 in CA. The incidence was highest among children < 1 year of age (178/100,000). Comparing data from the original FoodNet sites, overall incidence declined 6.4% from 1996 to 1998. This decrease was most evident in CA where there was a 34% decline. Declines were seen in the other four FoodNet sites from 1997 to 1998 ranging from a 5% decline in GA to a 15% decline in MN. Campylobacteriosis is a common illness. There is substantial geographic variation in incidence among the seven FoodNet sites with a decline in incidence in the original FoodNet sites from 1997 to 1998, and a sharp decline in incidence in CA from 1996 to 1998. To determine if this is due to changes in laboratory or clinical practices, FoodNet conducts ongoing surveys. Other possible reasons for this decline may be a reflection of ordinary variations in foodborne illness or a result of disease prevention efforts, such as changes in meat and poultry processing plants in the United States as mandated by the USDA Pathogen Reduction and Hazard Analysis and Critical Control Points (HACCP) Rule.

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CE29

## An Outbreak of *Campylobacter jejuni* Infections Associated with a Food Handler in an Elementary School

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**Background:** *Campylobacter* outbreaks are rare, and few have been associated with food handlers. In October 1998 we investigated an outbreak of *C. jejuni* infections at a school in Salina, Kansas, involving students, staff, and visitors who attended a luncheon on September 14, 1998; community cases were also reported. **Methods:** A case was defined as culture-confirmed *C. jejuni* or diarrhea (≥3 loose stools in 24-hour period) with onset after August 1, 1998, in a resident of Saline County. To identify risk factors for infection, we conducted a cohort study of 4th-6th graders, staff and visitors who ate lunch at School X on September 14, 1998. In the study, a case was defined as above with onset after September 14. Isolates were subtyped by pulsed-field gel electrophoresis (PFGE) and serotyped by the somatic **O** (Penner) method. **Results:** Overall we identified 27 culture-confirmed and 102 clinical cases in the county. In the cohort study, we interviewed 161 persons who ate the school lunch on September 14. Twenty-seven persons had illness that met the case definition; 8 (30%) were culture-confirmed. In the cohort study, 2 foods were associated with illness: gravy (RR=4.2, 95% CI=1.5, 11.7) and pineapple (RR=2.5, 95% CI=1.01, 5.8). This food was prepared in the school district kitchen and served to students in 6 other schools, where no illness was reported. A cafeteria worker at School X had diarrhea with onset on September 11 and worked while symptomatic during lunch on September 14. This person refilled food trays (e.g., pineapple) between classes, assisted children and cleaned tables, and was the likely source of food contamination. The PFGE pattern and serotype (**O**:19) from this person's isolate and 8 other isolates from persons who ate at that lunch were all indistinguishable. Isolates from 4 other *Campylobacter* patients in the county with illness during the same period had different patterns and serotypes. **Conclusions:** This outbreak of *C. jejuni* infections was associated with an ill food handler. PFGE was critical in identifying the cause of the outbreak and determining that community cases were not linked to the school. To prevent similar outbreaks in the future, persons with acute diarrhea should be excluded from handling food.

CE30

## The Prevalence of Potentially Neuropathic Strains of *Campylobacter* Within Tasmania Using the Cholera Toxin Binding Assay and Pulsed-Field Gel Electrophoresis.

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Prior infection with the bacterium *Campylobacter* has been implicated in the pathogenesis of Guillain Barré Syndrome (GBS), Miller Fisher Syndrome (MFS), acute motor axonal neuropathy (AMAN) and motor neuropathy with conduction block. The strains of *Campylobacter* implicated have been shown to contain lipopolysaccharide (LPS) epitopes within the core structure of the cell wall similar to a common epitope found in human peripheral nerves, GM1. As part of an epidemiological study into *Campylobacter* infection in Tasmania, 250 *Campylobacter* strains were isolated from faecal samples of patients presenting with gastroenteritis between October 1997 and February 1999. These isolates were typed by pulsed-field gel electrophoresis (PFGE) and the cholera toxin binding inhibition enzyme-linked immunosorbent assay for the detection of GM1 epitopes contained in bacterial cell wall LPS. Seventy-nine different restriction fragment length polymorphism (RFLP) patterns were found. Of these, 19 distinct RFLP patterns produced by strains were positive for GM1 comprising 33% of all isolates. No patient developed overt neurological disease. Using PFGE, no genetic similarity was seen between GM1 positive strains and control strains. PFGE and the cholera toxin binding inhibition ELISA can be used to determine which *Campylobacter* strains possess GM1 epitopes and therefore have the potential to induce neurological sequelae to simple diarrhoeal illness. Comparison with known pathogenic strains using PFGE may elicit which patients are at risk of developing neurological disease.

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CE31

## The incidence of antibodies to *Campylobacter* species in healthy blood donors, patients with previous campylobacter infections and patients with neurological disease

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Prior infection with Campylobacter has been implicated in the pathogenesis of the Guillain Barre Syndrome (GBS), the Miller Fisher Syndrome, acute motor axonal neuropathy and motor neuropathy with conduction block. As part of a large study into Campylobacter infections in Tasmania we measured the levels of antibodies to *Campylobacter* species in 413 healthy blood donors, 75 previously infected patients and 27 patients with neurological diseases. The aim of this study is to determine the relationship between acute and chronic peripheral nervous system disease and Campylobacter infection. The 74 patients with past Campylobacter infection, were patients who were notified as having Campylobacter in 1996 and who volunteered a blood sample. The assay used was an indirect enzyme linked immunosorbent assay developed utilizing a number of prevalent Tasmanian *Campylobacter* strains. A proportion of patients with prior Campylobacter infection have persisting antibody levels, as do patients with peripheral nervous system diseases. However, a causal link has not been established and is the focus of continuing work. Further analysis of immunoglobulin subclasses will be performed, as well as antibody assays on stored CSF and a comparison with anti-ganglioside levels.

## MOLECULAR EPIDEMIOLOGY OF CAMPYLOBACTER ENTERITIS IN QUEBEC: MARCH 1998 – FEBRUARY 1999 CE32

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4. Research Service, VA Medical Center, Boston University, Boston, MA

To determine the utility of molecular strain typing for a population-based collection of *Campylobacter enteritis* we used pulsed-field gel electrophoresis (PFGE) to analyze 154 isolates collected at the CUSE and at the CHUM Campus St-Luc between 1 Mar 98 and 28 Feb 99. Prospective epidemiologic data was available for 111 cases from Estrie representing 72 men and 39 women, with a median age of 25 years (range 10 months - 85 years). The principal suspected sources of infection were chicken (13%), raw milk (8%), and a foreign trip (9%); for 25% of cases the source was unknown and for 18% no data were available. The PFGE patterns of *Sma*I and *Kpn*I restriction were analyzed by computer software (GelCompar II, Applied Maths), using the UPGMA (unweighted pair group method with arithmetic averages) clustering method and the Dice similarity coefficient (SC). Sets of isolates with SC ≥ 90 % were considered closely genetically related. Among 149 isolates analyzed to date, PFGE of *Sma*I digests identified 17 sets of genetically related isolates that were also clustered in both time and space (from 2 to 4 patients each, for a total of 41 patients). To date 90 isolates have also been analyzed using *Kpn*I digests, with 77% agreement in the cluster assignment; the majority of disagreements represent isolates that had unique PFGE profiles by *Kpn*I but not *Sma*I. Similarly, a large group of 23 isolates with 70% SC by *Sma*I had only a 34% SC by *Kpn*I. These preliminary results indicate that *Kpn*I digests are more discriminatory than *Sma*I digests for the PFGE analysis of *C. jejuni* isolates. Complete molecular analysis of all isolates using *Kpn*I digests will be presented.

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## Ribosomal DNA Analysis Links a *Campylobacter jejuni* Illness to Washington State Shellfish Growing Area Reproted in 1993 CE33

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*Campylobacter* is found in various environmental and food sources, therefore, it is important to be able to trace the flow of the organism from its environmental source to the vehicle of infection. The first documented case of *Campylobacter* gastroenteritis associated with raw oyster consumption in the State of Washington was reported in 1993 (Abeyta et al.) Campylobacterosis occurred within 2 d of ingestion of a half-dozen shellstock Pacific oysters (*Crassostrea gigas*). Isolates were recovered from shellfish, seawater, and wild bird excreta from Penrose State Park located in a remote area and only open to the public on weekends. The park is not impacted by a high density residential area. Wild birds were observed in a pond caused by the high tide at the park. Samples were taken and prescribed diagnostic biochemical analyses were performed for phenotypic confirmation of *C. jejuni.* However, immediately after isolation, DNA genomic testing was not pursue to establish epidemiololgic relateness. New and simplified technologies for DNA testing within the last decade provide the food safety specialist epidemiological tools which can link cases of foodborne illness from the "farm to the table". Seven (7) isolates from environmental sources (shellfish, seawater, and bird excreta) were tested. All strains were indistinguishable by using ribotype patterns and that a common source was possible. Thus, water can be a source for trasmitting campylobacters from wild birds to shellfish.

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CE34 Identifiaction and differentiation Campylobacter jejuni and Campylobacter coli strains isolated from human and animals

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The method which is able to differentiate C. jejuni and C. coli was established by using PCR - REA method. For PCR reaction oligonucleotide primers from flagellin A gene previously described by Nuijten et al. 1990 were chosen. For futher confirmation amplified 410 bp fragments C.jejuni /coli were digested with the restriction endonuclease Hinf I./. Strains C. jejuni were not digested with Hinf I or were detected two fragments of which the molecular weights 110 and 300 bp. Two patterns were obtained for C. coli strains: type one had four restricted fragments /92, 18, 178 and 122 bp/, type two had three fragments /92, 196 and 122 bp/. These results suggested the ability to divide C. jejuni and C. coli by restriction fragment length polymorphism. About 90 % strains were typable by using digestion with the Hinf I. Strains C. jejuni from human showed mostly one profile type /two fragments/ and from chicken both types /two fragments and without digestion/. C. coli strains from human showed only type two /three fragments/ and C. coli from chicken both types /four and three fragments/.

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CE35 **Changing Morphology in *Campylobacter jejuni* Cells Cultured in Different Media.**

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In general, *Campylobacter jejuni* is cultured in rich, complex media such as BHI under micro-aerobic conditions. In this study, in addition to BHI, C. jejuni strains were grown in defined media supplemented with organic acids. Dependent on the composition of the defined media, remarkable differences in cell morphology or chain length were observed compared to that of BHI-grown cells. Some characteristics of the different types of cells were examined and will be discussed.

CE36

## Epidemiology and antimicrobial susceptibility of *Campylobacter fetus fetus* isolated in Québec, Canada, from 1983-1998

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Susceptibilities of 77 isolates of *C. fetus fetus* (*CFF*) to 8 antibiotics were studied by an agar dilution method. The antibiotics used were: ampicillin(AMP), cefotaxime(CTX),gentamicin(GT),ciprofloxacin(CIP),erythromycin(E), imipenem(IMP), meropenem(MER) and tetracycline(TET).The demographic and clinical information available at the LSPQ on the isolates recovered from 1983-1998 was also reviewed.

**Results**: Eigty- six *CFF* were isolated during this period, from a total of 79 patients, with a male to female ratio of 1.2 to 1. Seven patients had two isolates: 4 from different sites during the same time period, and 3 from the same site at different times, which could represent relapses. Clinical information was obtained for 75 patients. The number of infected patients varied annually between 3 to 11(mean and median = 7) with 62% aged≥ 60 years old. The isolation site was know for 67 patients: blood 69%, stool 20%, other 11% (aorta, CSF, joint, bile, ascite). Although bacteriemia accounted for the majority of infections, thirteen strains were isolated from stool only. Since campylobacter culture conditions in most laboratories are aimed at isolating *C. jejuni/coli* ( 42°C incubation and/or cephalotin containing media), *CFF* gastro- intestinal infection may be underestimated.

**Susceptibility testing results** (MIC = µg/ ml)

| | AMP | CTX | CIP | GT | E | IMP | MER | TET |
|---|---|---|---|---|---|---|---|---|
| $MIC_{50}$ | 2 | 8 | 0.5 | 0.5 | 1 | ≤0.06 | ≤0.06 | ≤0.06 |
| $MIC_{90}$ | 4 | 8 | 1 | 1 | 2 | ≤0.06 | 0.12 | ≥128 |

All isolates were susceptible to ampicillin, gentamicin, imipenem and meropenem. None were β- lactamase producer. Two isolates had intermediate susceptibility to ciprofloxacine. The overall tetracycline resistance rate was 36% with a significant increase in resistance noted during the years 1997- 98 (p=0.03).

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CE37

## Preliminary epidemiological studies on *Campylobacter* spp. in meat chickens in Queensland, Australia

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Management practices on Australian poultry farms differ significantly from those in Europe and the USA. As a first step to understanding the epidemiology of *Campylobacter* colonisation under local conditions, a cross-sectional study has been undertaken to determine the farm-level prevalence of *Campylobacter jejuni* and *C. coli* among meat chicken grower operations in South East Queensland. The survey was based on a formal random sample of all broiler grow-out farms supplying three major companies. All sheds on 56 farms were sampled at 28-35 days of age. Ten fresh faecal droppings per shed were collected and cultured on Karmali agar. At least one *Campylobacter* isolate from each positive sample was identified to species level. Of the 56 farms sampled, 26 (46.4%) were positive. Of the 239 sheds examined, 73 (30.5%) were positive. Of 589 positive faecal samples, 92% yielded *C. jejuni* and 7% yielded *C. coli*. Mixed infections with both species were demonstrated in 1% of samples. Univariable analysis of management practices suggested that re-use of litter from one production cycle to the next was significantly associated with *Campylobacter* colonisation (OR = 4.91, p = 0.02). This study has demonstrated that it is possible to produce some *Campylobacter*-negative poultry to the abattoir under Australian conditions. Longitudinal studies on selected farms combined with the use of DNA typing techniques will provide additional information on possible sources of the organism.

**CE38** **Evaluation of four methods for epidemiological typing of *Campylobacter jejuni***

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A number of different methods have been applied for typing of *C. jejuni*. Among the phenotypic methods the two serotyping systems, heat-stable and heat-labile serotyping, are the most common and discriminatory methods. The genotypic methods, such as ribotyping, pulsed-field gel electrophoresis (PFGE), flagellin gene typing (Fla-RFLP), restriction enzyme analysis, etc., are generally considered to be very discriminatory and have often been used for outbreak situations. In the present study four methods have been compared for typing of unrelated *C. jejuni* strains of human and broiler chicken origin.

A total of 65 *C. jejuni* strains were typed by the use of 'Penner serotyping' (heat-stable serotyping), flagellin gene typing using two restriction enzymes (Fla-RFLP), automated ribotyping using the RiboPrinter and pulsed-field gel electrophoresis using *SmaI* (PFGE). Forty-five clinical isolates were from sporadic cases of campylobacteriosis with no known relation to each other. Twenty strains were isolated from different broiler flocks.

The 65 strains were found to represent 19 different serotypes, 40 Fla-types, 40 ribogroups and 51 PFGE-types. PFGE was the most discriminatroy method (D= 0.96) and serotyping the least discriminatory (D= 0.81). Groups of isolates that had identical types by all four methods were found five times with two isolates in each group. One of these pairs of isolates represented different sources: a human and a broiler isolate. When only considering three of the four methods, several groups consisting of up to five strains were formed. In most of these cases, the serotyping, Fla-typing and riboprinting were in agreement whereas PFGE separated the strains.

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**CE39** **PFGE-DNA profiling of Danish *C. jejuni* serotype O:2 strains identify poultry, cattle and swine as reservoirs of sporadic human infection**

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*Campylobacter jejuni* is regarded as the most common bacterial cause of foodborne human gastroenteritis worldwide. Most cases are reported sporadically and the sources of such infection are rarely identified. A typing strategy for this purpose is thus required. We have observed previously that strains yielding the same PFGE-DNA profiles with each of four restriction enzymes (*Sma*I, *Sal*I, *Bam*HI, *Kpn*I) belong to the same serotype[1]. To determine more thoroughly the genetic diversity within a single serotype, we characterized 78 Danish *C. jejuni* serotype O:2 isolates (the most commonly encountered serotype in Denmark) from humans, poultry, cattle, and a pig by PFGE-DNA profiling using each of the aforementioned enzymes. A total of 42 different genotypes were recognized among the strains. Eleven groups ($n \geq 2$) of strains were obtained, of which seven contained isolates from a single source only (poultry or humans). Four groups of "genetically identical" isolates accounted for over 30% of the study population and were obtained from human, poultry, bovine and porcine samples. The stringent criteria used for strain identity clearly demonstrate that "genetically identical" clones from random sources can be identified, that relatively few clones of serotype O:2 may account for many cases of human illness caused by this serotype, and that poultry, cattle and pigs are sources of human infection.

[1] On et al. 1998. Epidemiol. Infect. 120: 231-237.

CE40

**Development of a simplified, agarose-based amplification fragment length polymorphism (AFLP) method for genomic typing of *Campylobacter jejuni***

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Amplification fragment length polymorphism (AFLP) has been described recently by two groups as a highly discriminatory technique for genotyping *Campylobacter jejuni*. Automated detection of fluorescently-labelled PCR products allows fingerprints of up to 60-70 bands to be resolved over a 50 to 500 bp size range. The aim of this study was to modify existing AFLP technology so as to generate simplified fingerprints of around 10-20 bands, these to be resolved by means of agarose gel electrophoresis. A restriction enzyme combination of *Bgl*II and *Mfe*I was found by fluorescent detection to generate fingerprints comprising less than 30 bands, after amplification with non-selective primers. This was considered to be a good starting point for development of a simplified method. Each of four selective *Mfe*I primers (with a one base addition at the 3'-end of A, T, C, or G) were synthesised and tested with the non-selective *Bgl*II primer against a pilot group of 10 *C. jejuni* strains. The number of bands generated was found to be primer-specific, with "C" and "G"-selective primers generating fewer bands (6-8) than the "A" and "T"-selective *Mfe*I primers (10-12 bands). Band sizes for all primer sets ranged between ~100 to 1500 bp, but "C" and "G" selective primers generated profiles which were biased towards higher molecular weights. "A" and "T" selective primers gave more evenly distributed profiles, allowing easier visual comparison between strains. The *Mfe*I-T selective primer was further tested on a sample group of 27 *C. jejuni* strains, including reference, outbreak- and genetically-characterised isolates. Indistinguishable profiles were generated from each group of outbreak or "genetically identical" strains, whereas unique profiles were generated from unrelated strains. This demonstrates the potential value of agarose-based AFLP for typing *C. jejuni* during routine epidemiological surveillance.

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CE41

Occurrence of Thermophilic *Campylobacter* Species in Macaque Colony in Brazil. LAURIA_FILGUEIRAS, A L.; ESTEVES,W.T.C.; SILVA, J.D.; VILARDO, M.C.B. & HOFER, E..

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*Campylobacter* infection in developing countries appears to have different clinical and epidemiological characteristics to that described for industrialized nations. Publications involving brazilian dates are unusual because only a few laboratories research these microorganisns in Brazil.

During the last three years we have isolated thermotolerant *Campylobacter* species from *Macaca mulatta* ( Rhesus monkey ), *Macaca fascicularis* (cynomolgus monkey) and *Saimiri* sp.

336 fecal samples were collected in 1996, 359 in 1997 and 386 in 1998 and we recovered *Campylobacter* in 33.63%, 28.69% and 33.68%, respectivally. *C. coli* biotype I was the specie and biotype more incidence and *Macaca fascicularis* monkey appear to be an ideal model for investigating this microorganism. None monkey presented the same result during the three collecting. A great number of *Campylobacter* sp was also detected, then we have to elucidation this problem, perhaps using molecular technics.

Only a few strains were sent to be serotyping by LCDC in Canada (27 from 1997 and 56 from 1998 isolates). As result, Lior serotype 29 was the most frequent but we had the same number of untypable strains with available antisera. So we have to do more investigation to check if they could be new serovares.

## CE42 Agreement of Probability Matrices for Identifying Campylobacteria

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The identification of campylobacters, helicobacters and related organisms belonging to the rRNA superfamily VI is complicated because of their slow growth, fastidious nature and biochemical inactivity. Probability matrices based on response to phenotypic tests incorporated in computer programs have allowed identification of these organisms. In this study two previously described matrices made up of 37 taxa, 67 tests (full) and 37 taxa, 18 tests (reduced) were evaluated for agreement in identification of 129 campylobacteria isolates using a computer program IDBACT. A simple algorithm was developed with the spreadsheet program, Microsoft Excel to interprete the agreement of the identification. The algorithm was based on addition of the decimal value (serial number) of the identification on the matrix to the identification confidence. Initial analyses suggest the matrices give total concordance for approximately 50% of test data, with minor errors detected in the confidence between identifications based on the two matrices for around 20% of strains. Both matrices agreed on identity of *Arcobacter* species but had varying results for thermophilic campylobacters and *H. pullorum*. There is need for a reduced matrix with perfect agreement with full matrix.

## CE43 Evaluation of a Neural Network for Identifying Campylobacteria

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The objective analysis of the results of phenotypic tests in a probability matrix is important to correctly assign a new isolate to a taxa in the matrix. This is a pattern matching problem. The bayesian probability approach has been used for the computer-assisted identification of campylobacteria. However, the need to use more robust data handling techniques is attractive. An artificial neural network (ANN) based on the backpropagation learning model was developed. The training set consisted on the probability matrix of 37 campylobacteria taxa and 67 phenotypic tests. Learning took 10 hours and a final error of 0.02 was observed. The final architecture consisted of three layers made up of 67-node input layer, 5-node hidden layer and 6-node output layer. The neural network was interrogated for ability to recognise the patterns of the matrix and the results of 129 strains with known identity. The ANN learnt 36 of the 37 patterns except that of *Bacteroides ureolyticus*. Furthermore only 5 out of the 129 results analysed agreed with the established bayesian statistics. Further studies are needed to clearly determine the potentials of neural networks in identification of campylobacteria. However, it is noted that the size of the identification matrix of is more extensive than those described for other groups of taxa.

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**Structural analysis of Post-translational Modifications of Campylobacter Flagellin.** CE44

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The flagellin of *Campylobacter coli* VC167 has been shown to display a serospecific glycosyl moiety as a post-translational modification. Mass spectrometric methods and carbohydrate analyses were used to characterize the chemical structure of these modifications. Nanoelectrospray analysis of intact flagellin from both parent VC167 cells and from a *ptm* mutant with altered antigenicity both produced flagellin of higher mass than that predicted from the translated gene product (approx 6,000 Da). The mass of flagellin from the *ptm* mutant was lower however than the parent confirming the involvement of post-translational modifications in antigenicity of VC167 flagellin. To elucidate the structure and location of these modifications, LC-MS and tandem mass spectrometry (MS-MS) analyses were conducted on the tryptic digest of flagellin from both VC167 and *ptm* mutant strains. Modified peptides were collected and subjected to further analyses using NMR spectroscopy. This presentation will describe the analytical strategy used to identify and characterize these unusual modifications and the sites of attachment.

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Application of spatial statistics to Campylobacter epidemiology CE45

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Campylobacter infection is the most prevalent bacterial gastrointestinal infection throughout the UK and Europe. Although large outbreaks are known to occur, most cases appear to be sporadic, i.e. the case is unrelated to other cases in time and space. Because outbreaks may sometimes be identified by current phenotypic and genotypic based typing schemes cases whose isolates have different typing results may be deemed unrelated. However, investigations on poultry suggest that a single carcass can carry several distinguishable strains of campylobacter. This may also be possible for other sources of campylobacter. Consequently, apparently sporadic cases may be infected by distinguishable strains that originate from a limited number, or single source(s). Point source outbreaks could be masked by this effect.

Campylobacter cases were collected from Central Southern UK over a 10 month period. Isolates were serotyped and risk factor questionnaires applied to each case. Time and space co-ordinates for cases with no evidence of foreign travel were derived from the date of onset and patient post code. Spatial statistics were applied to identify the extent of spatial and spatio-temporal clustering in the observed distribution of cases, and to investigate spatial variation in risk over the region.

CE46 **Prevalence and genomic diversity of *Campylobacter* (*C.*) *upsaliensis* in dogs and cats from two distinct regions in Germany**

NOTES

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*C. upsaliensis* is sporadically isolated from human disease, while it is often found in the intestines of dogs and cats. Its genetic characteristics and its possible pathogenic capacity in men and small animals are far from well defined [1]. To get further knowledge about this *Campylobacter* sp., a total of 262 dogs and 46 cats from two geographically different regions in Germany were investigated for the presence of *C. upsaliensis*. The isolates were characterized with respect to their species by biochemical tests and PCR [2], O-antigens [3] and genomic macrodiversity, which was assessed by restriction fragment length polymorphism with *Sma*I and *Xho*I and pulsed-field gel electrophoresis. A total of 109 strains was isolated. The prevalence of *C. upsaliensis* in dogs varied generally between 27 % and 42 %, irrespective of the geographic origin and the presence or absence of enteric disease of the hosts. In cats the prevalence was slightly lower (16 % - 22 %). 75.8 % of the *C. upsaliensis* isolates belonged to four different serotypes (preliminarily named I - IV), two of them (III and IV) being prevalent at 19 % - 45 %. Serotype II seemed to be a modified serotype I antigen exhibiting additionally crossreaction with the *C. jejuni* serotype O 2. Macrorestriction analysis revealed a considerable degree of genomic heterogeneity. No strict association was found when RFLP was compared to host species, geographic origin or serotype of the isolates, respectively.

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2. Eyers et al. (1993). J. Clin. Microbiol. 31: 3340-3343
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CE47 **A new genotyping system for *C. jejuni* strains based on LPS genes.**

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Subtyping of *C. jejuni* strains is an important tool to study epidemiology, however most of the phenotypic subtyping schemes have limitations with respect to discrimination power and applicability. Genetic subtyping schemes are also available but these do not fulfill the criteria that they correlate to the most commonly used Penner phenotyping scheme determining lipopolysaccharide (LPS) antigens; are able to identify strains that cause Guillain-Barré Syndrome; and are easy to use, generally applicable and result in stable genotypes. From the LPS serotyping it is evident that there is variation in the structures of the LPS molecules of *C. jejuni* strains. Since it is likely that this variation is reflected in the LPS synthesis genes, we investigated the genetic variation of the previously isolated LPS gene cluster (*wla*) of *C. jejuni* strains to design a genetic subtyping system that could meet all set criteria. PCR primers were designed for all 12 *wla* genes. Different Penner serotype strains were tested and amplification of all genes was confirmed. The PCR products were digested with restriction enzymes and the resulting fragments were compared to identify the degree of variation for all genes. Additional primers were constructed from conserved areas to flank a highly polymorphic region. The optimal combination of primers and restriction enzymes was selected to compare all serotype strains. Comparison of the obtained banding patterns showed a unique pattern for most of these. Strains sharing identical serotype were also tested to see if the selected genes were conserved within a serotype and to establish the correlation between LPS genotype and Penner serotype.

## Isolation of *Campylobacter* species from zoo animals and polymerase chain reaction-based random amplified polymorphism DNA analysis

CE48

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A total of 104 fecal specimens from 30 mammals, 12 birds, and 3 reptiles at Phoenix Zoological Gardens, Miyazaki City, Japan, was examined for the presence of *Campylobacter* species. All the animals examined were healthy with no disorders related to fecal conditions. Twenty-three (22.1%) thermophilic campylobacters, (9 *C. jejuni*, 11 *C. hyointestinalis* , 2 *C. coli* , and 1 *C. lari* ), were isolated from 11 animals (7 mammals and 4 birds). *C. jejuni* and *C. hyointestinalis* were the predominant species isolated from these zoo animals and *C. hyointestinalis* was isolated frequently from simians. For the epidemiological study, a polymerase chain reaction (PCR)-based randomly amplified polymorphic DNA (RAPD) method was used as a tool to detect the heterogeneity of amplified DNAs of *Campylobacter* spp. isolated from zoo animals. The two arbitrary primers used in this study enabled even closely related strains of the same *Campylobacter* spp. to be differentiated. RAPD analysis revealed considerable diversity among the strains, suggesting that the transmission of *Campylobacter* spp. among animals in a defined area occurred through different mechanisms. The experiment on the clonal relationships between single-colony isolates from an individual animal also revealed different banding patterns. These results demonstrate that campylobacter populations in zoo animals are highly divergent.

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## A Three-year Study of the Distribution of *Campylobacter jejuni coli* in Domestically Acquired Human Infections and Chicken Meat Samples from Helsinki Area.

CE49

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Campylobacter isolates from stool samples of enteritis patients with domestically acquired sporadic infections and campylobacters from chicken meat from retail shops were studied during seasonal peaks in June – September over a three-year period. There was a remarkable fluctuation in the number of human domestic campylobacter cases during the study period : 89 cases in 1996, 36 in 1997, and 74 in 1998. The respective percentageof positive meat samples from June to September was 32 % (17/52), 17.6% (37/206), and 20% (49/243). Human and chicken isolates were subtyped with PFGE using *Sma*I and *Sac*II restriction enzymes. A large number of genotypes were seen each year: 27 in 1996, 20 in 1997, and 27 in 1998. Certain genotypes persisted for the three year period, although the predominant genotypes were different each year. In 1996, two genotypes I/B and VIa +b + c covered 30.3% of the strains, whereas in 1998, 34.7% of the strains were covered by two genotypes, I/K and T101a + b. In 1997 no predominant genotypes were found. 17.6 to 20% of chicken meat samples were positive for campylobacters. A large variety of single genotypes were also identified among chicken isolates, and some of them were identical to those found in human infections.

**CE50** Concordant results in the comparison study of PFGE and AFLP patterns of predominant Finnish *Campylobacter jejuni* genotypes.

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During a three-year follow-up study on human and chicken campylobacter genotypes by PFGE pattern analysis (*Sma*I and *Sac*II), certain genotypes were shown to be common among strains from human patients and chicken meat samples. Within the selected PFGE genotypes, *Sma*I patterns were identical, and *Sac*II patterns were identical or closely related. AFLP, a new genotyping technique, was used to analyse the selected 35 *C. jejuni* strains isolated in 1997 and 1998 from humans and chickens. Ten strainsof PFGE type I/K, a common genotype in Helsinki area in summer 1998, shared the same AFLP type, further confirming the similarity of human and chicken strains. Similarly, 5 strains of PFGE genotype IV shared the same AFLP type, except one strain which was clustered with another AFLP type. Subtypes VIa, Vib and VIc which had different *Sma*I patterns but closely related *Sac*II patterns (2-3 fragment differences) were all clustered into one AFLP type confirming that these patterns are variants of one genotype and they form a lineage among Finnish *C. jejuni* strains. Also four strains of PFGE genotype T101, a new predominant type in summer 1998, showed an identical AFLP genotype. The present AFLP and PFGE genotype analysis indicates that PFGE and AFLP obtain similar level of differentiation and these methods can be used for studies on epidemiology and population genetics of *C.jejuni*.

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**CE51** **Molecular epidemiology of *Campylobacter jejuni* in Danish poultry flocks and in Danish wildlife**

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About 50% of Danish poultry flocks are infected with *C. jejuni*. Environmental sources are traditionally believed to be important reservoirs of *Campylobacter* infection in broiler flocks. We investigated this by comparing the serotypes and genotypes of *C. jejuni* strains from Danish broiler flocks with isolates from Danish wildlife (birds, ruminants and other mammals).

Forty five isolates from a wide range of wildliving animals isolated during 1996-98, and selected broiler strains from the same period were typed by Penner serotyping, PCR-Fla RFLP and MRP using PFGE.

Considerable serotype and genotype diversity was found within both reservoirs. The composition of sero- and PCR-Fla-types within the two reservoirs was not congruent, especially the wildlife collection contained serotypes that are less common in the poultry strains (O:12, O:38 and others), and O:1,44 isolates that constitute up to 18% of Danish broiler isolates were not found in wildlife. However some serotypes (O:2; O:4 complex) that together constitute more than 30% of isolates from the broiler production chain are also found in wildlife. MRPs (using restriction endonucleases SmaI and KpnI) seemed to indicate that, within those serogroups, a minor percentage of strains from wildlife belonged to clonal lines that were also found in broiler flocks.

Interestingly MRPs of O:12 and O:38 strains isolated from wildlife indicated that clonal lines propagated in a wide selection of animal species, whereas they have not yet been detected in the broiler production chain.

Our results indicate that the majority of clonal lineages detected in wildliving animals are not recovered from broiler flocks and vice versa. The typing methods used proved well suited for distinguishing between different clonal lineages within a strain collection showing large genomic diversity.

**A comparison of two *Fla*-typing systems for *Campylobacter jejuni*** CE52

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Two widely-used typing methods (Method 1; Nachamkin et al., 1993: Method 2: Ayling et al., 1996) based on the RFLP of PCR products of the *fla* genes have been compared. The methods vary in the primer sets, which either amplify *flaA* only or *flaA* and *flaB* together, and in the restriction enzymes used. *Dde*I was used in both methods, the other enzyme used was either *AluI* or *HinfI* respectively.

Fifty seven *C. jejuni* isolates from unrelated sources were investigated by both methods. Method 1 identified 33, whereas Method 2 identified only 27, distinct *fla*-types. One isolate was non-typeable in both methods. The *Dde*I profiles from each method produced similar results as expected. However the choice of the second enzyme contributed considerably to both the discriminatory power and the ease of interpretation.

Because the higher discrimination observed with Method 1 was often a reflection of minor band changes, the possibility that this was a consequence of intergenic recombination was investigated. Such rearrangements may not be observed using Method 2 which generates a mixture of fragments from both *fla* genes. Banding patterns from both the *flaA* and *flaB* PCR products were obtained independently using the primer sets from Method 2. As expected these results show that the *fla*A and *flaB* genes can have distinctively different RFLP profiles. Differences in discriminative power between the methods were not obviously explainable by intergenic rearrangements but appeared to be due to the difference in reverse primers and subsequent length of the *flaA* amplicon. In addition primer competition contributed differences in band intensities. The results indicate that the discriminatory power of *flaA/flaB* typing used in Method 2 may be made superior by amplification of *flaA* and *flaB* separately and by redesigning the *flaA* reverse primer to increase the PCR product size.

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*Campylobacter coli* Flagellin Types in a Single Litter of Pigs- Evidence of a Dynamic System. CE53

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Six piglets in a litter were sampled 6 times, using anal swabs, over the first 10 weeks of life. The sow was also sampled. Swabs were plated directly onto modified charcoal cefoperazone desoxycholate agar and purified campylobacters identified. On the first visit, three piglets had no detectable campylobacters but subsequently all piglets carried only *Campylobacter coli*. Five isolates per piglet, and 20 from the sow, were typed by PCR-RFLP (based on the *fla*A gene) after each visit. Visual examination showed that only 4 *fla* types (named A-D) were seen in the piglets over the period of study with type A dominating on 3 visits, C on two and B on one. All 3 were isolated from the mother on the first visit. Type D was detected 4 days after the litter had been exposed to another litter and swabs of 3 of those piglets revealed the presence of type D *C. coli*. Hence they probably introduced this type. After 10 weeks the piglets carried mainly A (83%) similar to the mothers initial flora. Prior to the second sampling, however, the mother died and the replacement foster-sow carried only *Campylobacter lari* which was completely replaced by *C. coli* after 21d. Hence piglets are rapidly colonised by *C. coli*, probably acquired from the mother, but sub-types are exchanged with the mother and with other piglets encountered with no stability of colonising types seen over the period studied. The sow sampled also showed no stability of sub-types emphasising the dynamic nature of colonisation.

**CE54** Effect of Isolation Temperature on RAPD Subtypes of *Campylobacter* spp. from Raw Poultry. P J SCATES, L MORAN and R H MADDEN Department of Agriculture for Northern Ireland Belfast BT9 5PX, Northern Ireland.

**NOTES**

*Campylobacter* species were isolated from retail packs of raw chicken (23) using enrichment in Preston broth incubated at both 37$^0$C and 42$^0$C in a microaerophilic atmosphere. The aim was to determine how the incubation temperature affected the types of campylobacters isolated. Overall 22 packs were positive, however 2 packs were only positive at 37$^0$C and a further 2 packs only positive at 42$^0$C. Two typical campylobacter colonies (A and B) from each enrichment were purified and biochemically identified as *Campylobacter jejuni* (96%) and *Campylobacter lari* (4%). One pack yielded *C. lari* with 2 isolates found at 37$^0$C and 1 at 42$^0$C. All campylobacter isolates from the 18 packs positive at both temperatures were subtyped by the random amplified polymorphic DNA (RAPD) technique using cell lysates. Profiles were analysed using Gelcompar (V 4.1) which revealed that only 2 packs had identical subtypes at both isolation temperatures. Overall 19 subtypes were found only at 37$^0$C and 22 only at 42$^0$C. This indicates that major differences in population subtypes selected arise as a consequence of the incubation temperature. Thus the isolation temperature appears to have a small effect on the species of campylobacter obtained from raw chicken. However detailed investigation reveals significant differences in the populations of sub-types obtained, indicating that samples are contaminated by a mixed population of sub-types. Hence to obtain representative isolates, especially in epidemiological investigations, the use of more than one isolation temperature should be considered.

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**CE55** **"Genotypic and Phenotypic Differences between *Campylobacter hyoilei* and *Campylobacter coli.***

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A PCR assay was developed to differentiate the porcine enteropathogen *C.hyoilei* from the closely related *C.coli* and *C.jejuni* species. An amplified DNA product of 383 bp was produced for all *C.hyoilei* strains tested. All other *Campylobacter* species, including *C.jejuni* and *C.coli*, and enteric bacteria tested showed no amplification product. The sensitivity of the PCR assay was 100 fg determined by using purified DNA. The detection limit in pure DNA could be increased by 10 to 100-fold after hybridisation of amplified DNA product with a $^{32}$P- labelled DNA probe containing an equivalent sequence. The sensitivity of the assay for detection of *C.hyoilei* cells was 200 cells/mL. The PCR assay was also used to detect *C.hyoilei* cells in faeces and the detection limit was 8.3x10$^4$ CFU/gm. This limit could be lowered to 2.1x10$^4$ CFU by Southern hybridisation. Thus, the detection limit of *C.hyoilei* in faeces was 105 CFU in 5µl of template before amplification. The findings indicate that the PCR assays developed in this study are suitable for detection and differentiation of *C.hyoilei* from other *Campylobacter* species, in particular *C.jejuni* and *C.coli*. Further evidence for the differences between *C.hyoilei* and *C.coli*, was obtained by measuring enzyme activities in strains of both species employing $^1$H- and 31P-NMR spectroscopy. Significant differences in the rates of enzymes involved in amino acid catabolism and synthesis, and phosphorus metabolism were found between the two species.

## Rapid Differentiation *C.jejuni* Isolates From Chickens And Humans Using PCR.

CE56

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Thermophilic *Campylobacter* species such as *C.jejuni* and *C.coli* are generally non pathogenic in animals but are recognised as major causes of acute gastroenteritis in humans world-wide. Contaminated foods, particularly poultry and minced meats, have been recognised as a major source of the disease in humans. We have developed a PCR assay based on a unique *ClaI* polymorphic site on the *C jejuni* chromosome to enable differentiation of C jejuni strains from chickens and humans. The PCR reaction resulted in amplification of 746 bp DNA fragment in all *C.jejuni* strains tested. The amplified DNA fragment could be cleaved using *Cla*I to produce a two band pattern in strains carrying the polymorphism. The DNA from other *Campylobacter* spp and enteric bacteria was not amplified using the same PCR test. The specificity and sensitivity of PCR test were determined for purified DNA, crude cell lysates, and faecal samples. The sensitivity of the PCR test was 25pgm of pure DNA, 3 x $10^3$ *C.jejuni* cells and $10^5$ cells/gm of faecal material. The PCR test included a second set of primers derived from a 16S RNA sequence specific for thermophilic campylobacters. This allows a single test to identify the presence of thermophilic campylobacters, differentiate *C.jejuni* from other species and group *C.jejuni* strains into two subgroups according to the *Cla*I polymorphism.

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## Direct Comparison of Pulsed Field Gel Electrophoresis (PFGE) and Riboprinter System® for genetic characterisation of *Campylobacter.*

CE57

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Several fingerprinting techniques which differentiate bacterial strains on the basis of small differences in their chromosomal DNA have been developed. In this study PFGE and ribotyping were directly compared. Ninety-six randomly selected *Campylobacter* strains isolated from poultry production and processing environment were typed using both techniques. For PFGE the rare-cutting restriction enzyme *SmaI* was used to generate restriction patterns containing a small number of large DNA fragments representing the complete bacterial genome. The Riboprinter Microbial Characterisation System®, an automated technique, commonly used for the rapid identification of bacteria to the sub-species level was also applied. This technique uses *PstI*, for the further chracterisation of *Campylobacter* and generates a larger number of DNA fragments followed by hybridisation with a non-radioactive labelled DNA probe. PFGE produced 8 genotypes within the 96 *Campylobacter* isolates investigated while ribotyping produced 5. One ribotype represented 4 unique PFGE types while the remaining four ribotypes represented the other four PFGE types. The 4 PFGE types which constitued one Ribogroup produced PFGE patterns which were closely related to each other. The automated Riboprinting system® was rapid, less labour intensive and allowed a greater throughput of strains.

CE58 **Evaluation of ProspecT Campylobacter Microplate Assay® for a 2 hours Detection of *C. jejuni* in Stool Specimens**

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**Introduction**. Isolation of *Campylobacter* spp in stools usually takes 2-5 days. With high and increasing levels of quinolone resistance in *Campylobacter,* empiric treatment of bacterial diarrhea with quinolones may result in treatment failures. Rapid detection of *Campylobacter* spp is therefore of clinical relevance. **Materials & Methods.**. ProspecT Campylobacter Microplate Assay® (Alexon-Trend, Inc) is a commercial ELISA, capturing campylobacters on the surface of a microplate well with polyclonal rabbit-anti-campylobacter antibodies. Total performance time is 2 hours. We tested 30 *C.jejuni* culture positive consecutive fecal samples upon arrival in the laboratory, after 1 week at 4 °C, 1 week at 4 °C in Cary & Blair (CB), and after 60 days at -20 °C. Thirty culture negative stool sampels were used as negative controls. **Results & Discussion.** In preliminary in-vitro experiments, using spiked fecal samples, the detection limit of the test appeared to be $10^4$ - $10^6$ CFU/gram depending on the strain. The sensitivities of ProspecT ® upon arrival, 1 week at 4 °C and 60 days at -20 °C were, resp., 80%, 80% and 77%. The sensitivity decreased to 37% after 1 week storage in CB at 4 °C. The specificity of the test was 100%. The ProspecT ® assay is a rapid, easy to perform test to detect *C. jejuni* in stools with satisfactory sensitivity and high specificity. Storage or transport of stool samples at 4 °C for 1 week or at -20 °C for at least 60 days, is possible without significant loss of sensitivity. The rapid 2h detection opens the way for early treatment options in the very young, the elderly and the immunocompromized patient. In addition, since *C.jejuni* related Guillain-Barré syndrome (GBS) patients have more severe variant of this disease we hypothesize that a positive ProspecT ® test result may serve as a marker for a more protracted and severe course of GBS. The performance of the assay in a historical collection of GBS feces stored at -20 °C is in process.

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CE59 **Isolation and Characteriazation of *Campylobacter* spp from sub-Antarctica**

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Birds and seals at Bird Island in the South Georgian archipelago were investigated for *Campylobacter* infection. In bird faeces, ten culture-positive samples (4,8%) were found in 1996, and six (1,3%) in 1998. No *Campylobacter* were found in the seal samples. The isolated strains were characterized by whole cell protein electrophoresis and phenotypic profiling. Representatives from different phenotypic groups were further characterized by sequencing of the *flaA* and/or *16S rRNA* genes, and for one group by PFGE.
The species *C. lari* and *C. jejuni* were identified which, to our knownledge, is the first report of these species in sub-Antarctica. The three *C. jejuni* isolates found were all from Macaroni penguins (*Eudyptes chrysolophus*) and had high phenotypic and genotypic homology to Northern Hemisphere strains. Six isolates could not be placed in either group, and were designated as *C. lari*-like.

CE60

**A novel method for Campylobacter geno-subtyping based on mutation analysis by denaturing gel electrophoresis.**

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Multiple genotyping methods for Campylobacter subtyping have been established such as PCR based RFLP, RAPD, DNA sequencing and whole genome PFGE. All have disadvantages of being either laborious or expensive in automated form. Complex band patterns can be difficult to reproduce.
The aim of this study was to apply electrophoresis in denaturing gels – so-called point-mutation analysis - as a new tool for bacterial genotyping. A PCR amplified *Campylobacter* flagellin gene fragment (702 bp) was selected as a polymorphic target gene. In denaturing gel electrophoresis the PCR amplicons of different *Campylobacter jejuni* migrated to different positions according to their melting point and change in structure after melting. Single base mutations are detectable. Similar migration patterns were obtained in denaturing gradient gel electrophoresis (DGGE), temporal temperature gradient gel electrophoresis (TTGE) and constant denaturing gel electrophoresis (CDGE). Ninety *C. jejuni* isolates revealed 21 serotypes and 15 homoduplex melting-point genotypes. Further discrimination was possible by heteroduplex analysis (HA) combining two genotypes or by combination of typing methods. The new PCR typing method needs no use of restriction enzymes, is a simple single band typing method and can easily be automated using capillary gel electrophoresis. Finally, this novel typing method has been compared to serotyping and 4 other genotyping methods. It is proposed as a simple and easy subtyping method for all Campylobacter species, but is also applicable to bacterial genotyping in general. Optimal condition for the analysis can be simulated from melting point computer programs.

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**Isolation of *Arcobacter* spp. from Naturally Contaminated Samples using Oxoid Arcobacter Enrichment Medium and Identification using SDS-PAGE and 16S rDNA Restriction Analysis.**

RE1

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In this study, the recently developed Oxoid Arcobacter Enrichment Medium (AEB) (Atabay and Corry, 1998) was evaluated using five samples each of minced pork, beef and lamb, five chicken carcasses and a 12 samples from a poultry processing plant environment. The samples were enriched statically, aerobically in Oxoid AEB at 30°C for 48h with tight caps, and then inoculated onto non-selective blood agar using the membrane filtration technique of Atabay and Corry (1997). Plates were incubated microaerobically at 30°C, examining daily after 2d, and thereafter for up to 5d. Isolates were identified using biochemical methods, SDS-PAGE of whole-cell proteins and 16S rDNA restriction analysis.

Arcobacters were detected in three of the pork, none of the lamb and only one of the beef samples. All the chicken carcasses and all the poultry processing plant samples, except for one from an eviscerator and both final wash water samples, were positive for *Arcobacter* species. The results of the two methods of speciation will be compared.

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CF1 **Transmission of *Campylobacter jejuni* from breeders to commercial broiler chickens**

NOTES

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We document breeder flocks as a source of *Campylobacter* for broilers. *Campylobacter jejuni* is a foodborne enteropathogen that has been closely associated with market poultry and is the most frequent agent of human gastroenteritis in the United States. The subject of transmission to the broiler flock has generated much discussion. Egg borne transmission from the parent breeder flock has been dismissed as a source of entry into the broiler flock because early studies were unable to recover *Campylobacter* from hatchery samples or from newly hatched chicks. In this study, fresh fecal droppings from a commercial broiler breeder (parent) flock were aseptically collected, placed into sterile centrifuge tubes, and transported to the laboratory packed in ice. The fecal samples were diluted in three times their weight of PBS and direct plated onto Campy-Cefex agar, incubated at 42°C (in a micro-aerobic environment of 5% $O_2$, 10% $CO_2$, 85% $N_2$). Isolated colonies characteristic of *Campylobacter* were examined by phase contrast microscopy for spiral shaped cellular morphology. A latex agglutination test was used for serologic confirmation. Fresh bird droppings were collected, using the same methods, from the progeny of the breeders. The progeny were a 6-week-old commercial broiler flock reared under typical United States husbandry practices 20 miles away from parent flock. *Campylobacter* isolates from these two independent flocks were characterized and compared by short variable region (SVR) *flaA* DNA sequencing. The results of the sequencing provided strong evidence that the isolates of *Campylobacter* were of clonal origin. In a second study isolates of *Campylobacter jejuni* from a parent flock were found to be of the same clonal origin as *Campylobacter jejuni* isolated from processed carcass rinse. This report provides the first cultural evidence that *Campylobacter* can pass from one generation to the next in broilers.

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CF2 **Prevalence of *C.jejuni* and *C.coli* in meat products and packaging sold at retail: A potential public health problem.**

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The objective of these studies was to determine the frequency and level of contamination of thermophilic campylobacters in raw meat products and on the outer packaging of these products sold at retail. On receipt at the laboratory the external surface of the packaging was sampled using a moistened swab and the meat contents were sampled by a rinse technique. The swabs and the rinse fluids were cultured using an established enrichment protocol. In addition, campylobacters were enumerated in the rinse fluid by a direct plating technique.
In the first study 136 samples of pre-packaged chicken portions were collected from 69 retail outlets. Eight-eight percent (120/136) of the poultry products and eight percent (11/116) of samples from the outer packaging of the products were positive for campylobacters.
In the second study 200 samples of chicken portions and 300 samples of offal (liver from three animal species) were collected from 300 retail outlets. Eight-five percent (170/200) of the poultry products and 3% (6/200) of samples from the outer packaging of the products were positive for campylobacters. Sixty-seven percent (200/300) of the offal products and 13/300 (4.5%) of samples from the outer packaging were positive for campylobacters. These results have obvious implications for the transmission of Campylobacter infections and raise several public health issues.

## A PCR-ELISA assay for the rapid detection of *Campylobacter jejuni* and *Campylobacter coli* in foods and environmental waters

CF3

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A rapid, sensitive and specific method for the detection of campylobacters in food and environmental samples is required to track campylobacter through the food chain and to further our understanding of the epidemiology of infection. There have been several reports of PCR methods for the detection of campylobacters in faeces, foods, and environmental waters. Sensitivity and specificity can be improved by using labelled probes specific for internal regions of the PCR target sequence in a colorimetric hybridisation format (PCR-ELISA). The aim of this study was to develop a PCR-ELISA assay based on a novel gene sequence (ORF-C) with species specific motifs. The suitability of four DNA extraction methods for the extraction of DNA from direct food samples and enrichment cultures was investigated and an optimised method devised. The optimised DNA extraction method and PCR-ELISA assay was applied to the detection of *Campylobacter* species in food and environmental water samples both directly and after 24 and 48 h enrichment culture. Results were compared with conventional enrichment culture methods. The PCR-ELISA assay was as sensitive as subculture to CCDA agar after 24 and 48 h enrichment culture (24h: PCR 48/77 +ve and culture 45/77 +ve; 48h: PCR 74/128 +ve and culture 80/128 +ve respectively) and was significantly more sensitive than conventional culture when applied directly to foods (PCR 40/115 +ve and culture 27/115 +ve respectively). The PCR-ELISA assay developed is robust, sensitive and specific for the detection of *Campylobacter jejuni* and *Campylobacter coli* in food and water samples and significantly reduces the time needed for detection.

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Survival and recovery of viable but non cultivable forms of *Campylobacter* in aqueous microcosm.

CF4

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Thermophilic campylobacters are the major cause of enteritis in industrialized countries and these infections are essentially due to poultry meat, non-treated water, raw milk and occasionally contact with pets. Thermophilic campylobacters can survive in untreated aqueous environments (stagnant water, distribution systems...) and could be a source of colonization of animals during breeding, or crossed contamination of carcasses during slaughtering and storage. This indicates that the survival of *Campylobacter* in aqueous microscosm is involved, directly or indirectly in human campylobacteriosis.

This study was realized on a collection of 87 strains of thermophilic campylobacters (*C. jejuni* and *C. coli*) in order to evaluate the capacity of survival and resuscitation of these strains. Differences were observed between the strains. The great majority are able to give viable but non cultivable forms after a stay of 30 days in an aqueous microcosm at 4°C. Two thirds of these strains were not detectable on agar medium after a stay of 14-21 days, 21% reached this stage before 14 days, and 11% were not cultivable after a stay of 21 days. Some strains remained cultivable in a shacked aqueous microcosm and beyond 60 days without shaking.

Assays of recovery on 9 days fertilized chicken eggs showed that 51% of the strains are able to revive and to grow. These results made in comparison with the classical method show clearly that some strains of thermophilic campylobacters are able to resuscitate and are potential risks of infections.

**CF5** Campylobacter contamination of poultry and carcass meats at retail sale and a comparison with isolates from human infection

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**NOTES**

*C. jejuni* is prevalent in the gastrointestinal tract of a wide variety of animals and thermophilic campylobacters have been isolated from a wide range of foods. This study investigated the incidence of campylobacters in bovine, ovine and porcine offal, and fresh chicken portions, sampled at retail outlets in two areas of the United Kingdom. In addition, serotype/phagetype profiles of *C. jejuni* from these samples were measured using the typing methods routinely used for reference typing of isolates from human infection.

A total of 894 meat samples were examined of which 489 [54.7%] were positive for campylobacter. Observed variation in contamination rates between laboratories may relate to laboratory methodology, types of retail outlet sampled or geographical variations in the incidence of campylobacter. Isolation rates also varied between sources with chicken being the most frequently contaminated followed by lamb and then pork. *C. jejuni* was the predominant species isolated from all sources but *C. coli* was prevalent in isolates from pork and *C. fetus* in beef. The distribution of *C. jejuni* serotypes in isolates from chicken and lamb was similar to that seen in concurrent human infections suggesting that both of these food sources may play a significant role in human infection.

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**CF6** A Quantitative Risk Assessment for Campylobacter in Broilers

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Quantitative risk assessments estimate the probability of unwanted events occurring. Such a model is currently being formulated which will assess the risks of human infection with campylobacter from the consumption of broiler products. With microbiological food-risk assessments in order to assess the risk to human health, it is necessary not only to estimate the probability of the organisms being present at each stage of the supply chain, but also the burden of organisms present at each stage.

Production and all other stages in the supply chain are of significance in relation to campylobacter contamination in poultry; therefore an investigatory process involving all stages of the supply chain is required to identify critical control points. To this aim all stages in the farm-to-fork poultry supply chain will be examined and at each stage, both the probability that any bird or food product is infected and the probable microbial dose will be estimated.

Construction of a set of GFP, YFP, and CFP promoter-probe vectors for use in *Campylobacter jejuni*. CF7

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The presence of *Campylobacter jejuni* on epithelial cell surfaces may be detected through the use of dyes or *Campylobacter*-specific antibodies. Each of these methods has various drawbacks: dyes may stain not only *Campylobacter* cells but other components of the normal microflora and antibodies may detect only a small subpopulation of the *Campylobacter* cells present on the epithelium. To improve the visualization of *C. jejuni*, we have developed a set of promoter-probe shuttle vectors that contain the *gfp* (green fluorescent protein), *yfp* (yellow fluorescent protein) or *cfp* (cyan fluorescent protein) reporter genes. These vectors are derived from pMW10 (Wosten *et al.*, J. Bacteriol., 1998, *180*:594-599) and contain both a *Campylobacter* and *E. coli* origin of replication, a kanamycin resistance gene and a reporter gene cassette that includes a pUC18 multi-cloning site (MCS) and transcriptional terminators 5' of the MCS and 3' of the reporter gene. Thus, random genomic fragments or small DNA fragments containing known promoter sequences can be cloned into the MCS.

To create a set of vectors, with a constitutively expressed reporter gene, that could be used to tag *C. jejuni* strains, a 45 bp fragment containing a "consensus" *C. jejuni* promoter ($P_c$) was cloned into the MCS of each vector. The stability of these vectors was tested by transforming two *C. jejuni* strains with a $P_c$-*gfp* fusion and subculturing each strain for nine days without selection. After nine days, ca. 100% of the cells retained the plasmid and >85% of the colonies were fluorescent. Cells containing these fusions are readily detected microscopically and have been used successfully in both poultry skin attachment and ligand binding studies.

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Exposures being protective for Campylobacter infection CF8

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Exposures more prevalent among controls than cases have traditionally been called protective factors. Risk factors, basis for interventions and control measures have generally been of most interest and their effect often proven in various studies – e.g. most of the risk factors identified in case control studies have been demonstrated as source of infections in different outbreaks. However, the number of studies evaluating and reporting on protective factors have been very limited. This paper presents results from a Danish case control study on sporadic campylobacteriosis and discusses hypotheses for the protective effect observed for different exposures. Eating unpeeled apples or pears in the two weeks prior to illness onset was associated with an odds ratio (OR)= 0.33 and p-value=0.0001. Eating offal from pork had an OR=0.24, p=0.005. The hypotheses being discussed are: systematic differences in recall bias between cases and controls, competitive exclusion of risk factor-foods with protective-factor foods, statistical coincidences and finally a real protective effect of the observed exposures. Epidemiological studies of sporadic campylobacteriosis during the last 15-20 years have not resulted in convincing intervention strategies or control measures. Recent years have broken new records in the registered number of human Campylobacter cases and it is time to focus on other investigation strategies towards preventing and controlling illnesses from Campylobacter infections – studying protective factors might produce useful insights.

CF9

# IDENTIFICATION OF CAMPYLOBACTER SPP. AND RESISTANCE TOWARDS ANTIBIOTICS IN PHEASANTS AND WILD GEESE SHOT IN GERMANY

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The economic importance of shot fowl is much less than that of domestic poultry, but even though fowl may carry all important bacteria pathogenic in food. Altogether 96 shot pheasants and 27 wild geeses from hunting areas from all over Germany were investigated microbiologically for Campylobacter.13.5% of the pheasants and 11.1% of the wild geese were positive for Campylobacter in this study. Different methods were used for their identification. All isolates of Campylobacter from pheasants and wild geese showed resistance towards antibiotics.

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CF10

**A Quantitative Risk Assessment Model for *C. jejuni* in Chicken**

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Quantitative risk assessment (QRA) is a tool that can be used to assist the decision-making process by providing a scientific and objective evaluation of the evidence. QRA involves mathematically modeling a food system, using data and expert opinion in order to arrive at an estimate of risk. The modeling process offers a unique opportunity to combine most of the data relevant to a specific issue in a manner that allows the entire system to be characterized.

A QRA for campylobacter in chicken was initiated to better understand the flow of *C. jejuni* on chickens from the farm to the consumer. The risk assessment model includes farm, transport, processing and consumer aspects of the "farm-to-fork" chain. Data from a variety of sources were collected and evaluated in order to characterize the individual stages of the "farm-to-fork" chain. Outputs from the model such as the concentration and prevalence of *C. jejuni* on chickens exiting the chill tank compared favorably with reported data. On average, 80% of the chickens exiting the chill tank were estimated to be contaminated with *C. jejuni*, and the average concentration was estimated to be approximately 4.5 log CFU/carcass. The risk assessment model identified the concentration of *C. jejuni* on chickens entering the process as an important determinant of risk, which implies that given current production and processing performance, steps taken to reduce the load and prevalence prior to slaughter would significantly reduce the risk to the consumer.

The presentation will illustrate QRA as an analysis and decision-making tool for microbial pathogens. In addition, the application of the *C. jejuni* risk assessment model to help identify potential risk intervention areas and data gaps in our knowledge will also be highlighted.

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Survival of *Campylobacter jejuni* on surfaces subject to drying. CF11

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Chicken carcases may be contaminated with large numbers of *Campylobacter* spp. and consequently undercooked chicken meat is a possible source of campylobacteriosis. There is also a potential risk of cross-contamination from raw chicken meat to ready-to-eat food in the domestic kitchen. *Campylobacters* have been recovered from kitchen surfaces on which raw chicken meat has been prepared. However, because of their sensitivity to drying, they are usually not expected to be culturable after the preparation area dries out.

In previous laboratory studies, using *Campylobacter* strains suspended in blood droplets, it was not possible to recover the bacteria once the droplets had dried. The present studies were undertaken to test the ability to recover *Campylobacter* spp., in improved enrichment broths, from surfaces in domestic kitchens and in model systems in the laboratory. Fresh chickens were prepared in either setting and surfaces on which the chickens were manipulated were swabbed at intervals. Some *C.jejuni* strains were used to spike samples of horse blood and chicken rinses, which were then dried on formica surfaces.

It was possible to recover *Campylobacter* spp. from naturally contaminated domestic kitchen surfaces 50 mins after the area was observably dry. From spiked horse blood droplets with an initial population of $10^5$ cfu the organisms were culturable at least 22 hours after drying . Results illustrating the roles of cell density, suspending medium and enrichment broths on the recovery of *Campylobacter* spp. from dried surfaces are discussed.

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**Influence of Feeding Patterns on the Presence of *Campylobacter jejuni* in Broilers Chickens.** CF12

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Feeding studies were conducted to determine the influence on *Campylobacter jejuni* colonization and detection in broiler chickens. In the first trial, 180 day of hatch chicks from two genetic strains were subjected to three feeding regimens: 1) full-fed, 2) meal-fed, and 3) skip-a-day. No birds tested positive via cloaca and ceca sampling for *Campylobacter jejuni* in this seven week trial. The second trial utilized 570 broiler chickens to consume a diet of all-plant or animal ingredients for a seven week period. Similar results were obtained, as both tested positive for *Campylobacter jejuni* at the same time and rate. The third trial utilized 1080 day of hatch broiler chickens that were subjected to a 0, 24, 48 and 72 hours delayed placement on used and unused litter. Chicks were ceca sampled weekly and via the crop at 49 days of age. No chicks tested positive for *Campylobacter jejuni* via the ceca or crop in any of the treatment groups in this trail. Results indicated poor colonization during the fall and winter when trial 1 and 3 were conducted. It was concluded that feeding stress and feeds do not influence the colonization or impact the subsequent detection of *Campylobacter jejuni* in the broiler growout environment.

**CF13** **Transmission of *Campylobacter jejuni* from breeders to commercial broiler chickens**

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We document breeder flocks as a source of *Campylobacter* for broilers. *Campylobacter jejuni* is a foodborne enteropathogen that has been closely associated with market poultry and is the most frequent agent of human gastroenteritis in the United States. The subject of transmission to the broiler flock has generated much discussion. Egg borne transmission from the parent breeder flock has been dismissed as a source of entry into the broiler flock because early studies were unable to recover *Campylobacter* from hatchery samples or from newly hatched chicks. In this study, fresh fecal droppings from a commercial broiler breeder (parent) flock were aseptically collected, placed into sterile centrifuge tubes, and transported to the laboratory packed in ice. The fecal samples were diluted in three times their weight of PBS and direct plated onto Campy-Cefex agar, incubated at 42°C (in a micro-aerobic environment of 5% $O_2$, 10% $CO_2$, 85% $N_2$). Isolated colonies characteristic of *Campylobacter* were examined by phase contrast microscopy for spiral shaped cellular morphology. A latex agglutination test was used for serologic confirmation. Fresh bird droppings were collected, using the same methods, from the progeny of the breeders. The progeny were a 6-week-old commercial broiler flock reared under typical United States husbandry practices 20 miles away from parent flock. *Campylobacter* isolates from these two independent flocks were characterized and compared by short variable region (SVR) *flaA* DNA sequencing. The results of the sequencing provided strong evidence that the isolates of *Campylobacter* were of clonal origin. In a second study isolates of *Campylobacter jejuni* from a parent flock were found to be of the same clonal origin as *Campylobacter jejuni* isolated from processed carcass rinse. This report provides the first cultural evidence that *Campylobacter* can pass from one generation to the next in broilers.

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**CF14** Development of a Selective Differential Agar for Isolation and Enumeration of *Campylobacter* spp. LINE, J.E., USDA, Russell Research Center, Athens, GA.

Direct plating is an effective technique for isolation and enumeration of campylobacters from a variety of sample types: however, distinguishing campylobacters from non-campylobacter contaminants which frequently grow on many existing agars is difficult. A more selective and differential agar was developed to facilitate *Campylobacter* enumeration by combining selective antibiotics and triphenyl-tetrazolium chloride (TTC). Growth of many microorganisms reduce the initially colorless tetrazolium salts to insoluble formazan compounds which impart a red color to the growing colonies. We found that exposing *Campylobacter* to low levels of TTC (200mg/L) was not inhibitory to growth, yet was sufficient to give a deep red/magenta color to the colonies. The new agar (Campy-Line agar, CLA) does not contain blood and is translucent. The contrast of deep red colonies on a translucent background greatly facilitates *Campylobacter* isolation and makes enumeration on light boxes or by electronic means possible. Direct plating of broiler carcass rinse samples (n=20) was compared on Campy-Cefex agar and CLA. Recovery of *Campylobacter* populations was not significantly different between the agars ($P<0.05$); however, isolation was much easier on the CLA. No contaminants were observed on the CLA, whereas the Cefex agar supported the growth of about 14 contaminating cfu/ml. In a separate trial, recovery of campylobacters from carcass rinses (n=25) was similarly compared. Again recovery of campylobacters was not significantly different between the two agars (Pearson correlation coefficient = 0.988) while about 9 contaminating cfu/ml were observed on Cefex agar and none on CLA. Experiments with pure isolates demonstrated CLA to support the isolation of *C. jejuni, C. coli* and *C. lari* strains from a background of common poultry contaminants. While some contaminants can still grow on CLA and can present red colonies, most of these contaminants are easily distinguished from *Campylobacter* by differences in colony morphology.

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**NOTES**

CF15

Reduced *Campylobacter* Populations Associated with Chickens Raised on Acidified Litter. LINE, J.E., USDA, Russell Research Center, Athens, GA.

Acidifying litter treatments have been demonstrated to reduce ammonia volatilization rates in poultry houses. Application of such compounds also reduces the litter pH and could affect pathogen populations in the litter. Two commercially available products, aluminium sulfate (alum) and sodium bisulfate, were tested to determine their effect on *Campylobacter* populations associated with broilers raised on treated litter. Fresh pine shavings served as litter in controlled isolation floor pens (49 $ft^2$). Broilers inoculated by oral gavage at day-of-hatch with a three strain mixture of campylobacters were grown at commercial densities in the pens for 3-6 weeks to contaminate the litter. These birds were then removed and the litter was immediately treated with two levels of alum (16 or 32 lbs/100 $ft^2$) or sodium bisulfate (5 or 8 lbs/100 $ft^2$). Untreated pens served as controls. Three replicate trials were conducted with duplicate treatment groups in each trial. Newly hatched chicks were released in the pens within 2 h of treatment. *Campylobacter* populations associated with ceca and whole carcass rinse samples (n=10) were determined for each duplicate pen at weeks 1, 4 and 6. Treatments were reapplied at week 5. The incidence of *Campylobacter* cecal colonization was significantly reduced in all trials by the high level alum treatment. Similarly, no *Campylobacter* was recovered from whole carcass rinse samples associated with these treated pens although control pens were 93, 72 and 32% positive at weeks 1, 4 and 6, respectively. The lower level alum treatment reduced cecal colonization frequency in 2 of the 3 trials while either level of sodium bisulfate treatment significantly reduced colonization in only 1 of the 3 replicate trials. Treatment of litter in poultry production may serve as a means to control *Campylobacter*.

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CF16

Comparison of Two Types of Plating Media for Detection and Enumeration of *Campylobacter* from Poultry Samples. BERRANG, M. E. and LINE, J. E. USDA, Russell Research Center, Athens GA.

A newly developed medium, Campy-Line agar, was compared to Campy-Cefex agar for recovery of *Campylobacter* spp. Broiler carcasses (n=18) were collected from a commercial processing plant. Five samples were examined from each carcass including: feathers, skin, crop, ceca and colon. All samples including surface contamination or internal contents were placed into sealable plastic bags, diluted and serial dilutions were plated onto each media. Campy-Line agar provided *Campylobacter* spp. counts that closely mirrored those found by enumeration on Campy-Cefex agar. Campy-Cefex agar generally provided slightly higher counts ($p \leq 0.05$) for all sample types except skin; however, compared to the means, the difference is slight. Expressed as a percentage of the mean count on Campy-Cefex agar, the differences between counts on the two plating media were 5% or less for each sample type and not likely to be microbiologically significant. Pearson correlations were conducted between counts recovered with the media. *Campylobacter* populations recovered with Campy-Line agar were correlated with those recovered using Campy-Cefex agar, r values were .94 for feathers, .95 for skin, .98 for crop, .87 for ceca and .88 for colon samples. Subjective observations suggest that Campy-Line agar is easier to use due to the virtual absence of contaminating colonies. These colonies can mask *Campylobacter,* making enumeration a challenging and tedious task. With the high level of correlation measured between counts from the two types of media, it is recommended that the ease of use of Campy-Line agar be taken into account when choosing a selective plating medium for *Campylobacter* recovery from a wide variety of poultry samples.

**CF17** Flow of *Campylobacter* spp. through U. S. poultry operations.

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The goal of the project was to determine the most significant sources of *Campylobacter* spp. in poultry operations. Almost 11,000 samples from 32 broiler flocks were quantitatively (for only feces and carcass rinses) and qualitatively evaluated. Genetic fingerprinting of the isolates is ongoing and will enhance identification of causal relationships. Prior to chicken placement, *Campylobacter* spp. was isolated from both mice and wild bird feces in or near the chicken house. Out of the hatchery, one of 775 paper pads tested yielded the organism. Insects, water lines and drinkers, litter, fan blade swabs, non-broiler animals on the farm, farmer's boot swabs and fly strips were positive only after the flocks became infected. After 6 weeks of production about 54%, and after 8 weeks about 96% were colonized at levels of $10^{5-8}$cfu/gm. Comparatively low frequencies (~30%) and levels (~$10^2$cfu *Campylobacter* spp. per carcass) were found. This reduction in public exposure (on the fully processed carcass) from previous frequencies approaching 100% and levels of ~$10^4$cfu *Campylobacter* spp. per carcass appears to have been accomplished primarily by increased application of chlorination in the chiller tanks. Among the four integrated cooperators, carcasses ranged from 21 to 41% positive. Difference in frequencies of positive infected excreta was observed in high (10.7%) vs low (7.7%) husbandry production efficiency, but this was considered as unimportant. Carcasses were positive at rates of 28% in spring, 45.7% in summer, 18.5% in fall and 24.2% in winter. Other potentially significant sources for broilers are described in another submitted paper by Cox and colleagues.

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**CF18** Uptake and Survival of Campylobacter jejuni in Pacific Shellstock Oysters

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Campylobacters have been found routinely in shellfish beds. Its transient existence in the marine environment primarily comes from wildbirds, farm runoffs, surface water and sewage bypasses. Shellfish are known to be potential carriers of pathogenic *Campylobacter* spp. Pacific shellstock oysters (*Crassostrea gigas*) were allowed to uptake 1 x $10^6$ cells/ml of *C. jejuni* for a period of 5 to 7 h in an artificial seawater aquarium system. Oysters were removed and stored at 4°C for 24 h. Uptake in oysters ranged from 0.4 to 114 cells/g. At intervals, oysters were removed and analyzed for *C. jejuni* by using Oxyrase for BrothTM (OB) and the FDA/Bacteriological Analytical Manual (BAM) standard methods. Comparison of these methods showed that the OB method is as reliable as the official FDA/BAM method. Survival of this pathogen in shellstock was notable, surviving up to 21 d. Depuration studies were explored to demonstrate attachment of the organism in oyster meats. Over time the levels of the organism were reduced, but not eliminated.

## Bloodless Media and Stationary Enrichment Dramatically Improve Cultural Success with Four Different Strains of *Campylobacter jejuni*

CF19

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This paper outlines a simple method for the culture of *Campylobacter* that produces superior growth compared to the standard method described in the Bacteriological Analytical Manual (BAM). The cultural study was initiated to look for alternate methods of culturing *Campylobacter* that do not require blood supplementation in either the broth enrichment or plate media. It was important that these techniques also be as sensitive as the standard method without becoming more complicated. The methodology employs 35$^{o}$ C. incubations in the standard *Campylobacter* gas mixture, stationary broth enrichments in 50 ml Erlenmeyer flasks, and bloodless broth and plate media. During the initiation of this study 50 ml Erlenmeyer flasks were found much more convenient than large enrichment vessels, and were used throughout. Subsequent experiments revealed that these flasks always produced greater numbers of bacteria than the standard stomacher bag enrichments. The data generated demonstrated that removal of blood from enrichment broth substantially increased culturability, if replaced by a supplement such as FBP. On the other hand, removal of blood from plate media, had little effect on the cultural outcome. Additionally, 50 ml Erlenmeyer flasks produced significantly greater growth then the standard enrichment vessel. These alterations in methodology represent enhancements in efficacy of culturability and ease of use as compared to conventional methodology.

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CF20

## Surveillance program on thermophilic Campylobacter spp. (C.jenuni, C.coli and C.lari) in raw meat products from Danish retail outlets.

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The result of a Danish surveillance program carried out by the Municipal Food Control Laboratories in Denmark 1995-1999 indicate that the prevalences of thermophilic Campylobacter spp. in broilers and turkeys at retail level in Denmark are in the range of 20 - 30% and 10 - 20%, respectively.From 1995-1999 the mean prevalences were 29% (N=1478) in broiler products of Danish origin, 33% (N=739) in broiler products, 23% (N=783) in turkey products of Danish origin, and 17% (N=367) in imported turkey products. The prevalences decreased during the sampling period, most significantly in turkey products of Danish origin. In poultry of Danish origin the prevalences were higher in summer months than in winter months. In frozen poultry products (20%,N=707) the prevalence was approximately 6% lower than in fresh poultry products (26%,N=443). In raw beef and pork products, 1% (N=1166) and 1.2% (N=1080) of the samples, respectively, were found to be contaminated with thermophilic Campylobacter spp. The result indicate that poultry products should be considered as one of the important risk factors regarding human campylobacteriosis. The relative importance of beef and pork products in relation to cases of sporadic human campylobacteriosis is to be investigated.

CF21

## Comparative Assessment of the Survival Rate of Campylobacter on Pig Carcasses after Blast Chilling and Batch Chilling

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Blast chilling is the most widespread system used in Danish abattoirs for pig carcass chilling. A comparative study is being carried out to assess the rate of inactivation of Campylobacter during blast chilling and batch chilling of pig carcasses. The samples are taken as surface swab samples covering areas of 1400 $cm^2$ from the pelvic duct, the hind leg and the cut face down to the breast bone including 5 cm rind.

The preliminary results indicate that blast chilling, where the carcasses pass through a chilling tunnel with a moderate air velocity at -22°C for 60 minutes followed by overnight equilibration at 5°C is superior to batch chilling with respect to the inactivation rate of Campylobacter.

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HF1

**Changes from bacillar to coccoid forms and culturability in *H. pylori* strains.**

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*H. pylori* is considered as an emerging food pathogen. Its role is presently speculative although circumstantial evidences suggest these bacteria would contaminate drinking water and sewage. *H. pylori* is a fastidious growing bacteria that easily reverts to coccal forms whose viability is controversial. The aim of this study was to prospectively evaluate culturability and the rate of bacillar to coccoid forms in three strains from different origins.

Two *H. pylori* strains were isolated from Chilean patients, one with gastric cancer (TC1) and the other with duodenal ulcers (TX30), the remaining strain was a type strain obtained from ATCC (43526). All strains were grown in Brucella broth supplemented with 0.5g/l of cyclodextrin during 24 days at 37°C under microarerophilic conditions without shaking. Morphologic changes were evaluated through microscopic observation and subcultures in each occasion.

| | Rate (%) of coccal forms | | | | |
|---|---|---|---|---|---|
| **Day** | **1** | **4** | **8** | **17** | **24** |
| **TC-1** | 0 | 20 | 10 | 30 | 5 |
| **TX-30** | 2 | 5 | 40 | 60 | 10 |
| **ATCC 43526** | 0 | 40 | 50 | 85 | 40 |

As observed in the table all three strains present different rates but similar patterns of coccal reversion, surprisingly enough no strain showed a complete reversion to coccal forms during the follow-up. Under this conditions the three strains remain culturable during the time studied. In addition strain TX30 sustained culturability for up to 18 days at 26° and 4°C in aerobic conditions, interestingly, only coccal forms were microscopically apparent. According to this data *H. pylori* would maintain viability under some stress conditions and eventually contaminate foods. FONDECYT 1990074.

**NOTES**

RF1

Distribution of *Campylobacter* and *Arcobacter* in Livestock.

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We have previously described PCR primers to distinguish *C. jejuni* from *C. coli* and to differentiate *Arcobacter* from other closely related species of *Campylobacter*. We have developed and applied these PCR-based methods to estimate the prevalence of these agents in healthy hogs and cattle and to correlate their distribution with on-farm management practices. To detect *Campylobacter*, 10% fecal suspensions were streaked onto modified mCCDA, incubated (48 h) microaerobically, and bacterial colonies were harvested into TE buffer for PCR analysis. To detect *Arcobacter*, 10% fecal suspensions were seeded into P-80 semisolid media (9 ml), incubated, and an aliquot removed for PCR analysis. In healthy pigs, *C. coli* (69 %), *Arcobacter* (46%), and *C. jejuni* (0.28%) were detected in 1,057 fecal samples. Of 1,334 cattle fecal samples analyzed, *C. jejuni* (43%), *Arcobacter* (11%), and *C. coli* (1.57%) were detected by PCR. These results indicate the widespread distribution of these microbes in healthy livestock.

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Comparison of Three Methods for the Isolation of Arcobacter spp. in Ground Pork

RF2

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*Arcobacter*, an aerotolerant organism similar to *Camplylobacter* has been studied recently due to its implication in foodborne illness. *Arcobacter* has been isolated from the food supply in two primary commodities, poultry and pork. The prevalence in pork has been found to be relatively low, typically 5%, using standard isolation methods. These methods include the Collins method (EMJH P-80 broth with CVA agar) and the Deboer method (ASB and ASM agar). In this experiment, these broth and agar systems were tested along with a relatively new method known as the Johnson-Murano (JM) method on the basis on sensitivity and specificity. Confirmation was carried out by PCR. The first objective was to test the sensitivity of each method by artificially inoculating raw ground pork with different levels of *A. butzleri* and *A. cryaerophilus*. The JM method was able to detect $10^1$ cfu/mL 100% of the time for *A. butzleri* and 75% of the time for *A. cryaerophilus*, while the Collins and Deboer methods were significantly less sensitive. The second objective aimed to determine the specificity of the three methods in detecting *Arcobacter* species in raw ground pork from processing plants across the United States. The JM method detected *Arcobacter* in 40% of samples from the East Coast, compared to <10% by the other methods. In samples taken from a plant in the Midwest, 68% of samples were positive for the organism by the JM method, compared with 10% by the other methods. Further testing will be done to gauge differences in prevalence of *Arcobacter* based on age of the slaughtered hogs and fat content of the ground pork.

CG1 MULTILOCUS ENZYME ELECTROPHORESIS ANALYSIS OF STRAINS OF UREASE-POSITIVE THERMOPHILIC CAMPYLOBACTER (UPTC)

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Recently the usefulness of multilocus enzyme electrophoresis (MEE) which has been employed for eukaryotic genetics was demonstrated for analysis of bacterial populations. Application of MEE analysis on population genetics of C. coli and C. jejuni was also reported. The present study set out to evaluate the use of MEE in analyzing the genetic structures of UPTC in our collection, as well as in England and in France and in determining the relationship between UPTC and C.lari. Since in initial screening of MEE analysis, any detectable staining profiles of 3 enzymes (ACO, IDH and THD) were not obtained for all strains examined, 7 enzymes (ADK, ALP, CAT, FUM, MDH, ME and PEP) among 10 enzymes which were reported to be useful for MEE of strains of C.coli and C. jejuni were used in this MEE analysis. Twenty nine strains of UPTC among 31 examined were grouped into 21 electrophoretic types, forming a distinctly different cluster from 3 strains of C. lari using 3 dimensional graphic blotted by the difference of allelic diversity. The dendrogram which demonstrated the genetic relatedness among strains examined was also constructed. The two strains examined using MEE analysis were demonstrated not to belong to the both clusters of UPTC and C. lari. Consequently, the present study suggest the existence of a genetical and biological barrier between typical C. lari and atypical C. lari, UPTC.

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CG2 STUDIES ON THE FLAGELLIN GENE OF UPTC AMPLIFIED BY USING PCR PRIMERS CONSTRUCTED FOR C. JEJUNI

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After the original description of UPTC from environmental sources in England, strains of UPTC were reported in France, in Ireland, in England, in the Netherland and in Japan, thus suggesting the worldwide distribution of this organism. Therefore, more extensive characterization may be required if we are fully to understand this organism. Recently, two flagellin genes of C. jejuni were cloned and sequenced, and the primers available for PCR amplification of the genes and the sequencing of the fragments amplified were reported. Therefore, an attempt was made to ascertain whether the analysis of flagellin gene of UPTC could be possible by using the system of the PCR. The present study set out to characterize the flagellin gene of UPTC by using the primer pairs.

A primer pair ($A_1/A_2$) ought to generate product of the expected size (about 1,720 bp) of flaA gene for C. jejuni amplified products of the size of about 1,450 bp for the 33 strains among the 44 strains of UPTC examined. The primer pair failed to amplify fragment for 11 strains of UPTC, 12 strains of C. lari and one strain of C. coli. Some other primer pairs (A5/A8; A5/A2; A1/A8) amplified products for some strains among the 11 strains.

Consequently, the fragment of flaA gene of UPTC amplified was demonstrated to be shorter than that of C. jejuni and the sequence around primer A1 and/or A2 of the flaA gene of some strains of UPTC examined may possibly be mutated. The DNA sequence of flaA gene fragment of UPTC will be demonstrated.

CG3

## CLONING AND SEQUENCING OF 16S rRNA GENES OF UREASE-POSITIVE THERMOPHILIC STRAINS OF *CAMPYLOBACTER*

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Sequencing of 16S rRNA genes (about 1,500 bp) of four strains of urease-positive thermophilic *Campylobacter* (UPTC) isolated from river water, human and seagulls was performed after PCR cloning. Moreover, sequence comparison among strains was performed in order to clarify the taxonomic situation and the relationships with other *Campylobacter*s.

The V2 variable region (equivalent to the nucleotide position from 126 to 276 of 16S rDNA in *E.coli*) of 16S rDNA of four UPTC strains was demonstrated to closely homologous (apporoximately 96% or more ) to those of *C. jejuni, C. lari* and *C. coli*, but not so homologous to those of the other species of *Campylobacter* examined. In relation to this point, the V2 variable region of *C. jejuni* and *C. coli* was demonstrated to be different from those of the four UPTCs at the 2 nucleotide positions. The region of UPTC showed to be identical with that of *C. lari*, except for the 2 nucleotide insertions for *C. lari*. The V 3 variable region (equivalent to the nucleotide position from 314 to 547 of 16S rDNA in *E. coli*) was shown to be indentical among those of four UPTCs and all the other *Campylobacter* species examined.

Distance-matrix analysis and dendrogram constructed for the estimation of the genetic relationships demonstrated that all the strains examined belong to the three major clusters, furthermore, the four strains of UPTC, *C, jejuni, C. lari* and *C. coli* forming one cluster. Comparison of phylogenetic relationships of these *Campylobacter* strains with strains from the other related genera will be demonstrated.

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CG4

## Is there a flagellar regulon in *Campylobacter jejuni*?

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The flagella of *Campylobacter jejuni* are among its best-characterised virulence factors. In other pathogens, flagellar expression is subject to regulatory control, for example in the Enterobacteriaceae by the master operon *flhCD*, controlled by catabolite repression among other factors, and the anti-sigma factor FlgM, but homologues of these genes are absent in *C. jejuni*. Recently, in the closely related *Helicobacter pylori*, a role for a regulator homologous to NtrC, designated FlgR, together with alternative sigma factor $\sigma^{54}$, has been demonstrated by others.

The availability of the genome sequence has made possible the identification of these and other potential regulators of flagellar gene expression in *C. jejuni*, and an analysis of genes potentially involved is presented. We have identified several candidates for this role, as follows. A gene Cj0355c resembling *ctrA* of *Caulobacter crescentus* is a probable transcriptional regulator, typical of two-component sensor-regulator systems although lacking a linked sensor gene, and is of interest in view of the role of its *C. crescentus* homologue in flagellar gene expression as well as cell cycle control. A gene Cj0466 resembling global regulators of the *crp* or *fnr* family might be involved in flagellar regulation by a mechanism resembling catabolite repression, albeit there is little other evidence of such a phenomenon in this organism. Also of interest are the *rpoN* gene Cj0670 encoding alternative sigma factor $\sigma^{54}$, known to be involved in flagellar gene expression; the *fliA* gene Cj0061c encoding $\sigma^{28}$; a gene Cj1024c homologous to the *ntrC/flgR* gene of *H. pylori*; and Cj0882c the *flhA/flbA* gene first reported by Miller *et al.* in 1993. A homologue of the latter has recently been shown to have a global regulatory role in *H. pylori* both among and beyond the flagellar genes.

Primers have been designed to amplify and/or modify the genes identified above, and constructs made for knock-out mutagenesis. Attempts to create knock-out mutants and investigate their phenotype are currently in progress.

**CG5** Cloning, sequencing, and expression of the *Campylobacter jejuni groESL* operon in response to stress.

**NOTES**

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The *GroESL* bicistronic operon from *Campylobacter jejuni* was cloned and sequenced. Sequence analysis revealed the presence of two complete open reading frames of 261bp and 1638bp which were identified as the *groES-* and *groEL-* homologous genes of *C. jejuni* respectively. The amino acid seqeunces of the two polypeptides encoded by these genes show a high degree of homology in comparison with respective proteins from other bacterial species.

Northern blot analysis revealed that transcription of the *groESL* operon was strongly heat-inducible. Western blot analysis using an anti-GroEL specific IgG also showed that expression of the GroEL protein was increased following heat shock and exposure of cells to various stress conditions such as UV, $H_2O_2$, cold, acid, alkali, and NaCl.

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**CG6** **Organisation, gene content and variation of the major lipopolysaccharide cluster of *C. jejuni* NCTC 11168**

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The complete genome sequence of *Campylobacter jejuni* NCTC 11168 has recently been made available (http://microbios1.mds.qmw.ac.uk/campylobacter/). The sequence is 1,641,480 bp in length and contains 1654 coding sequences. Analysis of the genome has revealed the presence of a cluster of 32 genes whose predicted protein sequences show similarity to known polysaccharide biosynthesis genes. Part of this cluster has already been characterised in our laboratory. The cluster contains the inner core biosynthesis genes *waaC, waaD, waaE, waaF, gmhA* and *galE*, and in addition three genes (*neuA*, *neuB* and *neuC*) thought to be responsible for the biosynthesis of sialic acid. Siaylation is a characteristic of *C. jejuni* core oligosaccharides.

The core oligosaccharide molecules produced by different strains of *C. jejuni* show differences in sugar composition, sequence and linkage. Studies on the *Enterobacteriacae* show that such structural differences often have a genetic basis. Using a simple PCR technique the gene content of this major lipopolysaccharide cluster was determined in several type and clinical isolates of *C. jejuni*. The results show that considerable genetic variation exists between different strains even between those of the same Penner serotype, highlighting the potential use of such an approach in typing or epidemiological studies. Several of these genetic polymorphisms have now been characterised by cloning and sequencing. The nature and significance of these polymorphisms will be discussed.

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**Expression of the *Campylobacter jejuni htrA* gene.** CG7

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In common with other bacteria, *Campylobacter jejuni* is likely to have a coordinated pattern of gene regulation in order to adapt to stress in the external environment. The stress response protein, HtrA, is involved in the adaptation to different environmental stresses such as temperature, osmotic and oxidative stress and has been identified in a variety of organisms, including *Salmonella typhimurium*, *Brucella abortus* and *Yersinia enterocolitica*. The *C. jejuni htrA* gene has been identified in previous work in our laboratory.

In the present study, emphasis was given to investigating expression of the *C. jejuni htrA* gene. The rationale for concentrating efforts in this direction stems from the fact that, to date, mutational analysis of the *htrA* gene has not revealed a phenotype. This could be due to a lack of expression of the protein in *C. jejuni*. Therefore, efforts were undertaken to develop a system whereby expression from the gene could be demonstrated and then subsequently be used to monitor expression under different environmental conditions. Furthermore, monitoring *htrA* expression will facilitate study of the genetic regulation of *htrA*.

In pursuit of this aim, two different approaches were adopted; i) a single copy chromosomal *htrA*::*cat* transcriptional fusion to measure *htrA* expression as a function of chloramphenicol acetyl transferase (CAT) activity and ii) the putative *htrA* promoter region cloned into pMW10 to investigate β-galactosidase activity as a measure of *htrA* expression. The data from these experiments show that *htrA* is expressed but, as yet, no signal has been identified that significantly increases expression.

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**Transcriptional organisation of the *fur* operon of *Campylobacter jejuni*.** CG8

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Iron is an essential nutrient for all living organisms as it is an important co-factor for some enzymes. However, in (micro)aerobic organisms free iron can create toxic oxygen metabolites. The concentration of free iron in the cytoplasm is controlled by the Ferric Uptake Regulator (Fur). This protein is a global regulator in Gram-negative bacteria, regulating iron acquisition genes, but also can be involved in regulation of genes encoding virulence and housekeeping functions. The transcription of the *Campylobacter jejuni fur* gene has been shown to have unusual characteristics when compared to other bacteria, as it is expressed on an mRNA which also contains the downstream housekeeping genes *lysS* and *glyA*. It has been suggested that the *C. jejuni fur* gene is the first gene in this operon and regulates its own expression.

In this study we demonstrate that the *C. jejuni fur* gene does not have its own promoter, unlike other bacterial *fur* genes. Upstream of *fur* we identified two Open Reading Frames (ORFs) of unknown function. Overlapping fragments containing putative promoters were tested using a *lacZ*-based reporter gene system. The transcription of *fur* was influenced both by the promoter of the gene directly upstream ($P_{ORF1}$), and by the promoter of the second upstream gene ($P_{ORF2}$). Expression from either promoter was not influenced by the environmental iron concentration. In conclusion, the *C. jejuni fur* gene is transcribed from two promoters in an operon also codeing for LysS, GlyA and one or two hypothetical proteins.

CG9 **Biochemical and Genetic Characterisation of *Campylobacter jejuni* Capsular Polysaccharide**

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*Campylobacter jejuni* lipopolysaccharide is thought to be involved in the pathogenesis of both uncomplicated infection and more serious neurological sequelae such as Miller-Fisher and Guillain-Barré syndromes. Current studies suggest that *C. jejuni* strains produce lipooligosaccharide (LOS) and about one third of strains also produce high molecular weight LPS referred to as O-antigen. In this report we demonstrate the presence of the high molecular weight LPS in all *C. jejuni* strains tested. Furthermore, we show that this LPS is biochemically and genetically unrelated to LOS and is similar to type II capsular polysaccharides. A gene cluster containing a number of genes (*kpsM, kpsT, kpsD, kpsE, kpsF, kpsC* and *kpsS*) similar to those involved in capsular polysaccharide biogenesis in *E. coli* was found. Three genes located at the distal ends of the cluster (*kpsM* at one end, and *kpsC* and *kpsS* at another) were chosen for insertional non-polar mutagenesis of seven *C. jejuni* strains of different serotypes. All 21 *kpsM, kpsS* and *kpsC* mutants constructed in this study lost their ability to produce O-antigen. Moreover, this correlated with serotype changes. We demonstrate for the first time that the previously described O-antigen of *C. jejuni* is a capsular polysaccharide and it is a common component of the thermostable antigen used for serotyping of *C. jejuni*.

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CG10 **NADH-quinone oxidoreductase in *Campylobacter jejuni***

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NADH-quinone oxidoreductase complex 1 (NDH-1) forms part the respiratory chain of aerobic organisms, transferring electrons from NADH into the quinone pool of the respiratory chain and translocating protons. Four NDH-1 complexes have been characterised to date, consisting of 14 non-identical subunits, encoded by a cluster of genes which occur in the same order. Critical to the functioning of the complex is the binding of NADH and the presence of Fe-S clusters. Subunit Nqo1 possesses the site for NADH binding, bound FMN and an Fe-S cluster, while Nqo2, 3, 6 and 9 have Fe-S clusters. The annotated genome of *Campylobacter jejuni* indicates the bacterium lacks genes encoding typical Nqo1 and Nqo2. In their place are two genes encoding polypeptides of a shorter length, which have very low homology to their counterparts and lack the conserved NADH and FMN binding regions, and the cysteines required to form Fe-S clusters. In addition, Nqo3 of *C. jejuni* contains an extra Fe-S cluster.

Searches using the Australian National Genomic Infromation Service, revealed fourteen fully or partially sequenced bacterial genomes possessing the genes encoding the components of the NDH-1 complex. The order of the genes in the genome is the same for most organisms, except for *Rickettsia prowazekii* in which the genes are scattered around the genome. Two species lack some of the genes encoding typical subunits; the partial genome of *Chlorobium tepidum* lacks *nqo1, 2* and *3*; and *Helicobacter pylori* lacks *nqo1* and 2, though interestingly, in place of these there are genes encoding polypeptides which show homology to their counterparts found in *C. jejuni*. In addition, *H. pylori* and *Aquifex aeolicus* have an extra Fe-S cluster in Nqo3.

These investigations showed that an unusual NDH-1 complex is present in the closely related microaerophiles *C. jejuni* and *H. pylori*. The physiological roles of these unusual complexes remain to be established.

## Survey of Internet Resources on Campylobacteria

CG11

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The Internet is a network of computers and it is becoming an important means of communication worldwide and specifically in biomedical research. The understanding of various aspects of the campylobacteria are extensive and emerging and the Internet offers enormous information. Where are these information located on the web? Using search engines queries were made for websites with title containing *Campylobacter*, *Helicobacter* or *Arcobacter*. A list of 16 web addresses was compiled including those of The 10th International Workshop on *Campylobacter*, *Helicobacter* and Related Organisms (www.med.upenn.edu/~campy10) (CHRO 10), complete genome sequences of *H. pylori* (http://www.tigr.org/tdb/mdb/hpdb/hpdb.html), *C. jejuni* (http://microbios1.mds.qmw.ac.uk/campylobacter/index.html). In addition, the home pages of campylobacteria researchers listed in the preliminary program of CHRO 10 are presented. The survey reveals key internet resources on campylobacteria, which can become starting point to obtain more detailed or specific information on the organisms. There is particularly a need to develop a website to track the emerging understanding of all aspects of campylobacteria research.

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## Construction and application of a *Campylobacter jejuni* DNA microarray to investigate differential gene expression.

CG12

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To cause disease, ***Campylobacter jejuni*** adapts to a range of stress-inducing environments through the differential regulation of gene expression in response to specific conditions. Recently the 1,641,510 bp of the *C. jejuni* 11168 genome has been sequenced at the Sangar Centre in Cambridge, UK. A DNA microarray for the entire *C. jejuni* genome has been constructed using clones from the ordered plasmid library which resulted from the genome sequencing, with the aim of quantitating mRNA expression of all genes in response to given stimuli. The microarray was prepared by the robotic printing of PCR products derived from each gene in the genome onto poly-L-lysine coated glass slides. Initial experiments have involved the exposure of *C. jejuni* to thermal, bile and aqueous stress. Total RNA was isolated from *C. jejuni* exposed to these stresses and from cells grown in broth as a standard control. By incorporating one fluorescent dye into cDNA synthesised from control RNA and another dye in cDNA from stress RNA, both these fluorescent cDNA probes can be hybridised to cognate elements on the microarray at the same time, allowing accurate measurement of the expression of the corresponding genes. Details of the construction of the DNA microarray and the analysis of differential gene expression in *C. jejuni* under these different environmental stresses will be presented.

CG13

## Identification of *Campylobacter jejuni* Virulence Genes by Insertion Mutagenesis

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Using the knowledge obtained on natural transformation of *Campylobacter jejuni* 81116 we have constructed a mutant library of this bacterium. The method involves insertion of a kanamycin-resistence ($Km^R$) cassette into a chromosomal library of *C. jejuni* DNA in shuttle vector pUOA18. *C. jejuni* was transformed with this library to allow homologous recombination between genomic fragments present in the shuttle vector and the chromosome. Chromosomal DNA from the pooled transformants was used to retransform *C. jejuni* resulting in transformants containing the $Km^R$ cassette in the chromosome but lacking the vector.
To evaluate the mutagenesis procedure the $Km^R$ insertional mutants were screened for loss of motility. Partial characterization of 11 non-motile mutants indicated that the inserted genes are involved in motility. Four mutants had the $Km^R$ cassette inserted in genes involved in flagella biosynthesis, namely *flaA/B*, *neuB* and *flgK*, and produced incomplete or no flagella. Four mutants had the $Km^R$ cassette inserted in genes possibly involved in flagella motor function: *pflA*, *fliM* and *orf1* downstream of the *fliN* gene. Three mutants had the $Km^R$ cassette inserted in genes that are homologous to genes encoding hypothetical proteins of *Helicobacter pylori*.
In order to identify genes involved in invasion, the mutant library was used to select mutants which were motile but non-invasive in an *in vitro* invasion assay with INT 407 cells. Genes possibly involved in the intracellular survival of *C. jejuni* may be identified by characterizing mutants which cannot survive in INT407 cells.

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CG14

## SEQUENCES DIVERSITY AMONG MOMPs AS A PARADIGM OF GENETIC VARIABILITY IN *CAMPYLOBACTER*.

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Fragments covering the entire gene of *C. jejuni* 85H Major Outer Membrane Protein were cloned into *E. coli*. The total aminoacid sequence was deduced from DNA sequencing and 100% homology was found with N-terminal sequence previously determined (Bolla et al 1995). Comparison of the sequences obtained in our laboratory with previously deposited sequences in libraries (Gene Bank accession numbers : AAC82317 and P80672) and with the MOMP from the strain NCTC 11168 obtained from the Sanger centre, showed interesting features:
The sequences are 90% homologous, the signal sequences are identical in the 3 sequences obtained by genetic analysis, the C terminal phenylalanine which is the signature of outer membrane porins, is conserved. Moreover, conserved sequences are delimited by variable regions where major differencescan be evidenced. As demonstrated previously for many porins:
-conserved regions are involved in structural organization, mainly antiparallel ß-sheets interacting with the outer membrane,
-variable regions, on the other hand, build loops exposed to the outer left of the bacteria, which contain specific recognition sites for phages, bacteriocins, and antibodies.
Taking into account the sequence comparison and previous structural results (Zhuang et al 1997) we present here a model for strategic regions of the MOMP of *Campylobacter*. The three dimensional study of the crystals recently obtained will certainly document this analysis.

CG15

Genomic rearrangement of *Campylobacter jejuni* after passage of chick intestine

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Our earlier studies revealed that rearrangements in the genomic structure of certain *Campylobacter jejuni* strains may happen during colonization in the chick intestine.In the recent study we inoculated 5 C. *jejuni* strains (PFGE type II/A, *Sma*I/*Sac*II) into one-day old chickens in order to study the frequency and time scale of the possible genetic rearrangements. Each stain was inoculated into ten birds and five of the birds were euthanized after 7 days colonization and further five birds were studied after two weeks. CFUs of *C.jejuni* were counted and 36 to 46 confirmed *C.jejuni* colonies from each group were randomly selected for genetic analysis. After one week, changed PFGE patterns were seen in two strains (BR 119 and BK 124), and after two weeks changed patterns were also seen in a third strain (FB 263). The frequency of changed colonies (PFGE, ribotyping ,*fla* typing) varied in different birds 5/5 (number of changed colonies/number of studied) to 1/5.For strain BR 119 4 birds of 9 had changed colonies and for strain FB 263, only 1of 10 birds had changed colonies. In the group inoculated with strain BK 124, all birds had a high frequence of changed colonies after one week but only one of five studied birds had changed colonies after two weeks. Our studies suggest that genomic stability of *C. jejuni* strains will vary. The changes will happen during primary colonization stage. The genomic change is most probably not a random but a regulated phenomenon.

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RG1

*Mucospirillum* gen.nov. –a new genus of spiral shaped bacteria colonising the mucus layer of the gastrointestinal tract.

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We have postulated that spiral morphology provides a selective advantage to bacteria that inhabit gastrointestinal mucus. Thus the lower bowel mucus of animals is colonised with large numbers of these bacteria mainly belonging to the genera *Helicobacter* or *Campylobacter*. Recently we have isolated bacteria that belonged to neither genus. These bacteria had a S-shaped morphology with bipolar unsheathed flagella and grew anaerobically on Campylobacter selective agar. Their whole-cell protein patterns differed from those of known *Campylobacter* and *Helicobacter* species. The 16S rDNA of a number of isolates was sequenced. This allowed phylogenetic analysis and the development of a PCR reaction that was specific for this group of organisms. These bacteria did not have any meaningful phylogenetic relationship to other intestinal organisms or to the class *Proteobacteria*. The most closely related bacterial species to this group of organisms was from the "Flexistipes" group, which forms a distinct lineage within the bacterial domain, one of over 30 phyla now recognized. While the "Flexistipes" group has been isolated from very diverse environments they all possess a spiral or curved morphology. We believe the present bacterium represents a previously undescribed bacterial genus for which we propose the name *Mucospirillum* with the type species *Mucospirillum schaedlerii* in honor of Russell Schaedler who was one of the early pioneers of the study of the bacteria of the intestinal tract of mammals and one of the first to isolate a member of this genera as part of the Altered Schaedler Flora.

**CP1** **Detection of cytolethal toxin activity and *cdt* genes in *Campylobacter jejuni* and *Campylobacter coli* isolated from Danish broiler**

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Campylobacteriosis in humans is mainly a food-borne infection and is one of the most important bacterial intestinal infections worldwide. Poultry and poultry products are considered to be sources playing an important role in transmission. In Denmark 30- 40% of poultry products have been reported *Campylobacter* contaminated at retail level and in other countries, prevalence between 2 and 100% have been reported. At present the pathogenesis is largely unknown, although a Cytolethal Distending toxin (CDT) has been incriminated as a virulence factor. CDT activity is conferred by the three adjacent genes *cdtA cdtB* and *cdtC*. This study was designed in order to investigate the prevalence of *cdt* genes in *Campylobacter* isolates from Danish broilers and their expression of the active CDT toxin. A total of 300 cloacal swabs were collected and tested by both conventional and PCR techniques. Of the 80 *Campylobacter* isolates that were detected, 72 were identified as *C. jejuni* (90%) and 8 were identified as *C. coli* (10%). Using PCR tests the *cdt* genes were detected from all of the isolates. Cytolethal toxic effects in African Green monkey kidney (Vero) and Chicken embryo cells were observed in 76 isolates. Active cytolethal toxin effects were observed in all *C. jejuni* strains. The *C. coli* isolates were produced little or no toxin. These primary results confirm the very common occurrence of *Campylobacter* infection in single chicken and indicate that the *cdt* genes may be commonly present in *C. jejuni* and *C. coli* isolates from broilers, whereas the active cytolethal toxin effects may differ between the two species.

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**CP2** **Characterization of Epithelial Cell Receptor of *Campylobacter* in Transfected Chinese Ovary Cells Expressing Human H-2 Determinants.**

**CERVANTES LE.**[1], NEWBURG D.S.[2], PRIETO P.[3], RUIZ-PALACIOS G.M.[1].
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*Campylobacter* infection is one of the most common causes of diarrhea around the world. One essential step for infection by enteropathogens is attachment to mucosal surfaces; however, the bacterial adhesins to HEp-2 cells and the corresponding host's receptors have not been defined in *Campylobacter* infection. We have shown that *Campylobacter* binding is inhibited by receptor analogs of human milk containing fucosylated oligosaccharides (OS), which are genetically determined and related to the mother's blood group type. Blood group antigens are expressed in intestinal mucosa and could function as coreceptors. To elucidate the molecular structure of the cell receptor for *Campylobacter*, we analyzed 9 neoglycoproteins corresponding to the determinants H-2, H-1, and Lewis by Western blot (WB) with a prototype strain of *C. jejuni*; inhibition assays were done with specific monoclonal antibodies (MAbs) to the different blood group antigens. Bacteria-receptor docking was demonstrated by bacterial association assays to CHO cells transfected with the human α1-2fucosyltransferase gene, and by inhibition assays of bacterial binding by H-2 ligands and homologs in transfected cells. Inhibition of the bacteria-receptor recognition was observed in WB, mainly with the anti-H-2 MAb. Association index of *C. jejuni* to transfected CHO cells reached 100%, while cell association with parental CHO cells was lower than 10%; a significant inhibition was observed with anti-H-2 MAb (91.5%), the lectins UEA I (84.3%), and TP (61%). In the competitive assays, the association index of *C. jejuni* decreased 100% with human milk OS, 85.7% with the neoglycoprotein H-2, and 82.1% with 2'fucosyllactose. Results suggest that H-2 (O) blood/tissue group antigens play an important role in attachment of *C. jejuni* to the intestinal mucosa, and could be related to susceptibility to *Campylobacter* infections.

**Identification of H-2 (O) Determinants in Human Intestine as Receptor of Campylobacter.** CP3

**Ramos-Cervantes P**, Cervantes LE, Ruiz-Palacios G.M. National Institute of Nutrition, Mexico City, Mexico.

Pathogenesis of *Campylobacter* diarrhea involves a series of complex events in which adherence to gut mucosa, mediated by recognition between bacterial adhesins and intestinal receptors, are of primary importance.
*In vitro* studies have established that *C. jejuni* is able to adhere to different cell lines, but significant difference in the association index has been observed between cell lines. Epithelial cells grown *in vitro* may express receptors different than in normal intestine. Because of this, it is important to define the association of *C. jejuni* to normal intestinal tissue, as a first step in the characterization of *C. jejuni* receptor.
We evaluated the adherence of *C. jejuni* to paraffin-embedded tissue from human ileum, cecum and colon to determine the site of grater adherence.
*Ex vivo* studies of *Campylobacter* infection in fresh intestinal biopsies were standarized first in ileum and cecum of rats and studies of infection and inhibition of infection in human intestinal tissue were further set up using human milk and synthetic fucosylated oligosaccharides (OS).
In paraffin-embedded tissue, a significantly greater adherence of *Campylobacter* was observed in cecum, followed by distant ileum and descending colon.
Adherence to fresh human intestine was consistent with an average colonization of $10^3$ per $cm^2$ . 90% and 64% reduction of tissue colonization were observed by preincubation of *Campylobacter* with milk OS and 2'fucosyllactose, respectively.
The inhibition of adherence by human milk OS and 2'fucosyllactose suggests that the biochemical nature of the *C. jejuni* receptor is the same as that of the O (H) blood group antigen expressed in the intestine by gene regulation of FUT 2 (Se).

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***flaA* and *flaB* Fingerprint of *C. jejuni* Strains and their Relation with Virulence Characteristics.** CP4 **RAMOS -CERVANTES P.** CARVALHO A.C.T., CERVANTES LE, RUIZ-PALACIOS G.M. National Institute of Nutrition, Mexico City, Mexico.

The mechanism by which *Campylobacter* causes illnes are not complety understood, altough colonization to intestinal mucosa is considered an important mechanism of pathogenicity. This microorganism posseses a polar flagellum composed of two flagellin subunits encoded by the *flaA* and *flaB* genes. It confers high motility that is considered an important feature to establish infection. PCR-RFLP of the *fla* gene can be used as an epidemiological marker.Using RAPD technique, we identified a 1.6 kb virulence marker, *iam* gene, related with *Campylobacter* association to Hep-2 cells present in 84% of invasive strains. This gene has four different *Hind*III, H1, seeing mainly in pathogenic strains, H2A, H2B, present in non-pathogenic strains, and H2C.
The aim of this study was to determine if there is an association between the RAPD pattern of the flagellum with the presence of *iam* gene and its restriction patterns. 110 strains of Campylobacter, classified as invasive or non-invasive as determine by the Hep-2 cells association assay evaluated, were evaluated by PCR-RFLP. The *flaA* and *flaB* genes were amplified with specific primers and digested with *Eco*RI and *Pst*I. The electrophoretic patterns were analized by GelCompar program for generating and plotting a dendogram. We identified 10 clusters of RFLP patterns. Four of this clusters were associated with the presence of the *iam* gene, the restriction pattern H1 and with Hep-2 cell association . One group was negative for the prescence of the *iam* gene, cell association and the strains presented the restriction pattern H2B. A good correlation between PCR-RFLP fingerprint and genotypic and phenotypic characteristics was observed.

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CP5 **Mutation in the *iamA* Locus of Invasive *Campylobacter jejuni* Reduces the Ability to Adhere and Invade HEp-2 Epithelial Cells.** **CARVALHO A.C.T.**, JUAREZ D, TORRES A, RAMOS-CERVANTES P, CERVANTES LE, RUIZ-PALACIOS G.M. National Institute of Nutrition, Mexico City, Mexico.

*C.jejuni* is one of the main etiologic agents of diarrhea worldwide. Adherence to and invasion of epithelial cells has been considered as one of the main virulence factors. In a study in Mexico we observed that the most *C. jejuni* strains isolated from patients with inflammatory diarrhea were invasive, while most *C. jejuni* strains from asymptomatic children were non-invasive. Using the RAPD fingerprinting method to study invasive and non-invasive strains, we were able to identify a new locus, named invasive associated marker (*iam*). An internal fragment of 518bp of *iamA* was amplified in 84% of the invasive strains and in only 22% of the non-invasive strains. IamA shared amino acid identities with several ABC transporters and IamB with some hydrophobic hypotethical proteins. To determine the role of *iam* locus in adherence and invasion of *C. jejuni* to HE-2 cells, three mutants with a disruption in *iamA* locus with Kanamycin and Chloramphenicol gene cassettes from *C. coli* were generated by allelic exchange into *Hind*III, *Eco*47III and *Eco*47III/*Hind*III sites. The phenotype of adherence was evaluated in HEp-2 cells and in α1-2 fucosyl transferase transfected cells for each mutant and the wild type strain. A 90% reduction of cell association was observed in the mutant strain when compared with wild type strain 287IP,. In this study, by using different gene cassettes, *the iam A* was disrupted in different sites, producing several mutants lacking the ability to invade epithelial cells, demostrating that *iamA* is an essential gene for invasion.

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CP6 ***iam* Locus is a Genetic Marker Strongly Associated in *Campylobacter jejuni* with the Phenotype of Adherence to and Invasion of HEp-2 Epithelial Cells.** **CARVALHO A.C.T** ,RAMOS-CERVANTES P, X. JIANG, CERVANTES LE, L. K. PICKERING, RUIZ-PALACIOS G. M.. National Institute of Nutrition, Mexico City, Mexico and Center for Pediatric Research, Norfolk, USA.

Cell adherence and invasion has been considered a major pathogenic mechanism of *Campylobacter* infection. In a study in Mexico, 70% of *C. jejuni* strains from children with diarrhea were invasive, while 83% of isolates from asymptomatic children were non-invasive. By RAPD techniques we observed that most invasive isolates (43/70; 61%), compared with those non-invasive (8/49; 16%), have a 1.6-Kb band ($X^2 = 23.74$, $P$=.0000011). This genetic marker was named ***Campylobacter* invasive associated marker** (CIAM). The CIAM was cloned and sequenced; the 1.6-Kb band revealed a total of 1630 pb. Sequence analysis indicated the presence of an ORF, named *iamA* (nucleotides 303-1022), which encoded a putative protein of 239 amino acids. The sequence upstream of *iamA* suggested the presence of a second incomplete ORF, *iamB*. The beginning of *iamB* was not detected, but a partial 133 amino acid residue was identified. IamA protein shared identities and similarities with several ATP binding cassettes. A pair of specific primers, internal to the *iamA* gene, were designed to amplify a 518bp PCR product. The expected PCR product was amplified in 84% (59/70) of invasive strains, whereas only 22% (11/49) of the non-invasive strains was positive ($X^2$=45.12; $P$=.00000000). A numerical analysis of the RAPD fingerprinting was carried out using the GelCompar software. Two main clusters (I and II) were identified, exhibiting a low level of similarity. Cluster I comprised most non-invasive strains and the CIAM and *iamA*-PCR negative strains, and the majority of invasive strains were in Cluster II. In both clusters we identified subgroups with undistinguishable RAPD patterns, characteristics of clonal related organisms. In this study, we were able to identify a genetic marker strongly associated with the phenotype of adherence to and invasion of epithelial cells.

CP7

Polymorphism in the major outer membrane protein in *Campylobacter jejuni*

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Polymorphism in the MOMP of *C. jejuni* was reported by Newell *et al.* in 1984, but its molecular genetics has not been investigated. The MOMP has been implicated in pathogenesis-related functions including adhesion and toxic activity.

We have investigated MOMP expression by SDS-PAGE, in a range of isolates of *C. jejuni*. These included well-characterised laboratory strains (81116/NCTC 11828 and the genome sequence strain NCTC 11186), as well as isolates from the environment, broiler houses and poultry carcasses for consumption. Widespread polymorphism was observed among this randomly assembled group of 16 strains, with apparent molecular weights with at least 6 different values ranging from 41 to 46 kDa. Few other variations in major proteins were observed. To assess the extent of genetic polymorphism and provide material for further characterisation, PCR primers were designed, based on the genome sequence (Sanger Centre) to amplify the entire MOMP gene and several hundred base pairs of flanking sequences. The MOMP gene appeared monocistronic, with significant intergenic regions flanking it upstream and downstream. These primers readily amplified sequences from many, but not all, of this group of strains. Some size variation was seen in the amplicon. Attempts to clone this large PCR fragment were not successful. Additional primers were designed to amplify the sequence in two segments, representing the 5' and 3' parts of the gene, again with flanking sequences. Again, amplification was variable and there were indications of size heterogeneity. Cloning of these fragments has been successful only for some of the 3' fragments to date, and these are currently being characterised further.

These data indicate that there is gene sequence variation as well as extensive apparent molecular mass polymorphism among MOMPs of *C. jejuni*. Further clues to the function of the protein may follow from definition of the molecular basis for the polymorphism.

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CP8

Characterisation of expression of flagellar gene *flhB* in *Campylobacter jejuni*

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Regulation of expression of flagellar genes in *C. jejuni* appears to differ significantly from that of well-characterised organisms such as *Salmonella typhimurium*. In contrast to the latter, it appears from the genome sequence that *C. jejuni* lacks key regulatory genes *flhCD* and *flgM*. A homologue of *flhB*, believed to control hook length and export of flagellar components, which we have previously cloned and characterised from *C. jejuni*, is therefore an important candidate for a regulatory role in flagellar expression.

Studies of *flhB* expression are now reported. The gene appears to be monocistronic, being downstream of *ahpC* and separated from it by a putative terminator sequence. Downstream of *flhB* is a convergent *motB* homologue. Transcription of *flhB* has been characterised by primer extension which showed that the transcriptional start is not associated with a putative consensus promoter-like sequence previously identified, but rather at a location about 100 bp upstream of the *flhB* start codon, within the proposed transcriptional terminator sequence of *ahpC*.

To determine the size of the *flhB* transcript and the extent of transcription of *flhB*, northern blotting was attempted but no transcript could be detected, suggesting a very low level of transcription. Control northern blots for *flaA* expression indicated that transcription of the latter is reduced although not totally repressed in *flhB* mutants. In view of the near-total cessation of FlaA expression in *flhB* mutants, this suggests that there may be an element of translational control in FlaA expression. Constructs were also made in the shuttle promoter probe vector pMW10, to investigate levels of expression of B-galactosidase in *C. jejuni* under the control of *ahpC* and *flhB* promoter regions. These experiments confirmed that levels of *flhB* transcription from the promoter region identified by primer extension were extremely low. No relation of expression to growth cycle stages could be detected.

**CP9** Hyperplastic Gastritis in scid Mice Infected with *Campylobacter fetus*. V.B. YOUNG, C.A. DANGLER, J.G. FOX, and D.B. SCHAUER. MIT, Cambridge, MA 02139.

We have developed a scid mouse model of infection with *C. fetus* that is characterized by significant gastric pathology. *Helicobacter*-free 4-week-old ICR and ICR scid mice were orally inoculated with *C. fetus*. All inoculated mice became persistently infected, but no clinical signs of disease were noted. At 1 month in both ICR and scid mice spiral bacteria were seen in the distal antrum, often closely opposed to the epithelium. The accompanying antral hyperplasia and inflammation was somewhat more severe and extensive in scid mice than in ICR mice. By 3 months scid mice had marked antral hyperplasia and inflammation. Two of 5 scid mice also had inflammation and parietal cell loss in the fundus. In contrast, ICR mice still had only mild inflammation in the distal antrum. *C. fetus* also colonized the cecum and colon, but intestinal lesions were not noted. We have developed a murine model of persistent infection with *C. fetus* that features pathology similar to that seen in mice infected with gastric *Helicobacter* spp. The finding of gastric pathology was unexpected because *C. fetus* is generally associated with lower bowel disease.

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**CP10** *Campylobacter* Enteritis : An Italian Working Group For Quality In Diagnosis And Surveillance

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*Campylobacter* is one of the most common agents of acute enteritis in Italy too, in children particularly. Some clinical aspects are mandatory for its prompt identification. Some increased resistances, to quinolones f.i., suggest a suitable surveillance of these infections. Italian Health System provides for investigation, identification and antimicrobial sensitivity tests. Nevertheless, a recent enquiry of AMCLI pointed out various gaps in the screening and diagnosis. So we have organized a working group on *Campylobacter* infections: CAMPYG. The main aims are education for standarized diagnostic procedures and specific monitoring through strains and data collection, supported by ISS too. Further aims are contact with european study groups and interaction with veterinary area for better understanding the epidemiology of this zoonosis in our country. We started with two basic formation courses, a presentation at Industry Meeting and publishing a monography on *Campylobacter* and related genera infections.

## Mutation and phenotypic analysis of the *waaF* genes of *C. jejuni* NCTC 11168 & 81116

CP11

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Analysis of the complete genome sequence of *Campylobacter jejuni* NCTC 11168 has revealed the presence of a coding sequence with a high level of similarity to the *waaF* genes of other Gram negative bacteria. In *Escherichia coli* the *waaF* gene encodes a heptosyltransferase which adds a second heptose to the inner core moiety of lipopolysaccharide. Using PCR methodology the putative *waaF* genes from two strains of *C. jejuni* were cloned. The strains used were NCTC 11168 which is reported to produce no O-chain and belongs to Penner group 2, and 81116 which does produce O-chain and belongs to Penner group 6. The cloned genes were deleted using inverse PCR and replaced by a chloramphenicol acetyl transferase gene (CAT). Following allelic exchange of the mutated alleles into the chromosome of the homologous *C. jejuni* strain, phenotypic analysis was undertaken. This investigation revealed that both the C. *jejuni* NCTC 11168 and 81116 *waaF* mutants produced a truncated core oligosaccharide as judged by SDS-PAGE followed by silver staining and Western Hybridisation using serotyping antisereum. This observation supports the identification of the coding sequence as *waaF*. Another important observation was that the O-chain of the 81116 *waaF* mutant remained unaffected both in mobility or intensity compared to the wild-type as judged by Western Hybridisation. The potential importance of this result to our understanding of lipopolysaccharide structure in *C. jejuni* will be discussed.

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## Characterisation of the haemin uptake system of *Campylobacter jejuni.*

CP12

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In the host environment, the essential nutrient iron is sequestered by host iron-binding and iron-storage proteins, thus acting as a form of non specific host defence against colonisation by pathogenic organisms. Pathogens have evolved specific proteins to obtain iron in from the host environment. Haemin uptake by *Campylobacter jejuni* is likely to play a significant role during the colonisation and pathogenesis of the host. It has been shown previously, that strains that do not express a 70kDa outer membrane protein are unable to utilise haemin or haemoglobin as the sole iron source. This 70kDa protein, designated ChuA, is iron- and Fur regulated and shows sequence similarity to haem receptors from other bacteria. The *chuA* gene is located in an operon which also contains three downstream genes, designated -*chuB*, *chuC* and *chuD*, which encode an ABC transporter system.

The function of the *chuABCD* proteins was investigated by insertional mutagenesis of *chuA, B, C* and *D* in *C.jejuni* strain NCTC11168. These mutants were assayed for the ability to utilise haemin as the sole iron source. The *chuA* mutant was unable to utilise haemin as the sole iron source showing that loss of the outer membrane receptor prevents haemin uptake. In contrast mutants in *chuB, chuC* or *chuD* were still able to utilise haemin as effectively as the wild type strain. In conclusion, the *C.jejuni chuABCD* operon encodes a haemin uptake system, and the mutants created will allow assessment of the role of this system in colonisation and pathogenesis by *C.jejuni.*

CP13 Surface structures and a putative capsule in *Campylobacter jejuni* NOTES

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Knowledge of the surface structure of *C. jejuni* is important for a complete understanding of both the molecular and biochemical basis of any serotyping scheme. Surface exposed antigens also have an important role as pathogenicity determinants. The evidence for the production of classical long-chain lipopolysaccharide [LPS] in *C.jejuni* is equivocal, and we have been unable to demonstrate LPS using either silver stain or immunoblotting under conditions used for other enteric pathogens. We have demonstrated that the heat-stable antigen is both resistant to proteolytic digestion and easily eluted from the cell surface. Because of these findings it has been previously suggested that the serotyping scheme is based on variations in a capsular structure. We have now further clarified this by specific staining of a high molecular weight acidic carbohydrate, the use of immunogold labelling of paired isolates differing only in a deletion mutation in a single gene and analysis of a purified carbohydrate component of a strain of *C. jejuni*. The results of this work will be presented.

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CP14 **Signal Transduction Events Essential for *Campylobacter jejuni* 81-176 Invasion of Epithelial Cells. L. HU and D. J. KOPECKO, FDA-CBER, Bethesda, MD 20892**

The molecular mechanisms of *C. jejuni* pathogenesis are not well understood, but intestinal invasion appears to be an important early step. Similar to other invasive intestinal pathogens, *C. jejuni* colonize the intestine, trigger specific host signal transduction events, and cause host cytoskeletal rearrangements that result in bacterial internalization. Gentamicin-kill, host cell invasion assays were employed to assess the effect of various inhibitors on bacterial entry. *C. jejuni* strain 81-176 entry into INT407 cells was blocked by monolayer pretreatment with inhibitors of: protein kinases(genistein and staurosporine), PI-3 kinase(wortmannin and LY294002) and caveolae formation(filipin III). Inhibitors of protein phosphatases were also analyzed. 100 nM okadaic acid caused only a slight reduction in *C. jejuni* entry, but 1 nM calyculin reduced entry by ~80%, suggesting that host protein dephosphorylation is essential for bacterial entry. Changes in host cell free-$Ca^{++}$ levels have been shown to be important at various stages of microbial infection, including bacterial invasion. $Ca^{++}$ chelators were used to pretreat human INT407 intestinal cells prior to bacterial invasion assays. Extracellular $Ca^{++}$ chelators did not affect bacterial entry. BAPTA-AM, a host cell-permeable $Ca^{++}$ chelator, blocked *C. jejuni* 81-176 invasion by 90%, and 50 μm dantrolene, which inhibits $Ca^{++}$ release specifically from the sarcoplasmic reticulum, reduced bacterial entry by 50%. Using the $Ca^{++}$ -sensitive dye Fluo 3-AM in INT407 cells revealed a time-dependent, early elevation of intracellular free $Ca^{++}$ after infection with *C. jejuni*, relative to control cells. Collectively, these data suggest that *C. jejuni* may interact with putative receptors in host membrane caveolae. A signal transduction cascade is stimulated in the host cell, which results in specific tyrosine-phosphorylation, serine-threonine phosphorylation or dephosphorylation of 9 host proteins, identified by immunoblotting studies. Specific activation of PI-3 kinase leads to $Ca^{++}$ release from intracellular stores, which is likely required for the host cytoskeletal rearrangements that result in bacterial internalization.

**CP15**

***Campylobacter jejuni* 81-176 Specifically Associates with Microtubules and Dynein and Migrates Perinuclearly During Controlled Invasion into Human Intestinal Cells.** **L HU and D. J. KOPECKO, FDA-CBER, Bethesda, MD 20892**

The pathophysiology of diarrheal disease caused by *Campylobacter* spp. is poorly understood, but current evidence suggests that, at least, some *Campylobacter* spp. cause invasive intestinal disease. *C. jejuni* 81-176 enters cultured human intestinal cells via a bacterial strain-dependent process that requires polymerized microtubules, but is unaffected by depolymerization of microfilaments. In the current studies, *Campylobacter* uptake into cultured INT407 cells was kinetically analyzed over a wide range of starting multiplicities of infection (MOI; i.e. from 0.02 to 20,000 bacteria/epithelial cell). The invasion process was apparently saturated in 2 hours at a MOI=200, and ~2 bacteria entered per infected host cell, indicating stringent host cell limitations on this entry process. However, only two-thirds of monolayer cells were infected 2 hr. postinfection, even at MOI's of 200-2000. This number of infected host cells gradually increased to 85% after a 7 hr. infection, suggesting that bacterial entry may be cell cycle-dependent. Direct evidence of the involvement of microtubules in *C. jejuni* internalization, verified by biochemical inhibitor studies in Caco-2 cells, was obtained by time-course immunofluorescence microscopic analyses. At early times during bacterial invasion, *C. jejuni* are observed interacting with finger-like extensions of the cell membrane containing 1 or a few bundled MT's, which may represent an early host cell cytoskeletal response to a signal from the nearby bacterium. *C. jejuni* were observed to align in parallel with microtubules during entry, were found to co-localize specifically with microtubules(MTs) but not with microfilaments, and moved over 4 hr. to the perinuclear region of host cells. Orthovanadate, which inhibits dynein activity, markedly reduced *C. jejuni* 81-176 entry, and invading *C. jejuni* colocalized with dynein, suggesting that this molecular motor is involved in both *C. jejuni* entry and endosome-trafficking during this novel bacterial internalization process. We hypothesize that *C. jejuni* interact with a receptor in membrane caveolae, activating membrane-bound dynein, whose movement along MTs may aid in the membrane invagination event that results in bacterial entry.

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**CP16**

*Campylobacter fetus sap* inversion can occur in the absence of RecA function. KC RAY[1]; SA THOMPSON[2]; ZC TU[1]; R GROGONO-THOMAS[3], DG NEWELL[4], MJ BLASER[1] 1) Vanderbilt University School of Medicine and VA Medical Center, Nashville, TN 2) Medical College of Georgia, Augusta, GA 3) Royal Veterinary College, London, UK 4) Veterinary Laboratories Agency, Surrey, UK

A surface layer contributes to the virulence of *C. fetus*. Phase-variation of *C. fetus* surface layer proteins (SLPs) occurs by inversion of a 6.2 kb DNA segment, permitting the expression of 1 of 8 SLP-encoding genes. Unlike phase-variation systems of other bacteria, in which inversion occurs independent of RecA function, previous work (J. Bacteriol. 1997; 179: 7523) showed that the *C. fetus* inversion system is *recA*-dependent. However, a *recA* mutant (created by insertion of the *aphA* kanamycin–resistance gene) of wild-type *C. fetus* 23D that expressed the 97 kDa SLP (97-211) was inoculated into a pregnant ewe and then cultured from the vagina and placenta. One of the strains cultured from the vagina (97-210) expressed the 97-kDa protein, as expected. However, a strain (97-209) recovered from the placenta expressed a 127 kDa SLP, an indication that chromosomal rearrangement may have occurred. We therefore sought to characterize *C. fetus* 23D (control), 97-211, 97-210, and 97-209, and to determine whether rearrangements had occurred. In previous studies, *C. fetus* cells that were deficient in RecA function were highly sensitive to the mutagenizing agent methyl-methane sulfonate (MMS). Examination of the strains used in this study indicated MMS resistance for wild-type strain 23D, whereas strains 97-211, 97-210, and 97-209 were highly sensitive to MMS, indicating their lack of RecA function. Southern hybridization and PCR of these strains indicated that the *aphA* insertion into *recA* was present and stable. Southern hybridization also demonstrated that a rearrangement event had taken place in strain 97-209. PCR data further confirmed rearrangement of the 6.2 Kb DNA element. These results indicate that DNA inversion in *C. fetus* may occur independent of RecA function. The relative frequency of *recA*-dependent and –independent inversion is not known.

**CP17** *Campylobacter fetus* SapC is not required for SLP expression, secretion, or antigenic variation. SA THOMPSON, KC RAY, MH BEINS, ZC TU, and MJ BLASER. Medical College of Georgia, Augusta, GA, and Vanderbilt University, Nashville, TN

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A surface-layer (S-layer) contributes to the invasive properties of *Campylobacter fetus* by conferring resistance to phagocytosis and to serum bactericidal activity. *C. fetus* uses a type I secretion apparatus to secrete surface layer proteins (SLPs), the subunits of the S-layer. In all other type I secretion systems, the apparatus is composed of three proteins homologous to *C. fetus* SapDEF. These proteins are expressed from genes at the distal end of a putative operon (*sapCDEF*) located in the 6 kb invertible element between *sapA* and *sapA2* in strain 23D. Previous studies showed that a *C. fetus sapD* mutant is unable to transport SLPs to the cell surface, resulting in the absence of an S-layer and serum sensitivity. Although probably co-transcribed with *sapDEF*, the product of the *sapC* gene is predicted to not form a component of the transport apparatus. To investigate the function of SapC, a non-polar insertional mutation was created in *sapC* that should abrogate its function. The *sapC* mutant expressed wild-type levels of SLPs, and they were secreted normally to the cell surface. Wild-type serum-resistance of the *sapC* mutant indicated the presence of a functional S-layer. Finally, the DNA inversion events among the SLP-encoding genes that are responsible for the antigenic variation of SLPs continued to occur in the *sapC* mutant. Therefore, SapC is not required for SLP expression, secretion, or in DNA inversions resulting in antigenic variation, and its function remains unknown.

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**CP18** *C. fetus* uses multiple loci within the 5' conserved region of *sap* homologs for DNA inversion. ZC TU, KC RAY, SA THOMPSON, AND MJ BLASER. Vanderbilt University School of Medicine and VA Medical Center, Nashville, TN, and Medical College of Georgia, Augusta, GA.

*Campylobacter fetus* has 7-8 promoterless *sap* homologs, each capable of encoding a surface layer protein (SLP), that can be expressed by utilizing a unique promoter present on a 6.2kb invertible element. Each of 3 cloned *sap* homologs (*sapA*, *sapA1*, *sapA2*), and 3 other genes (*sapA3*, *sapA4*, and *sapA5*; unpublished data) possess a 626bp conserved 5' region, (74bp upstream of the ORF, and 552bp within the ORF). The 6.2kb invertible element is flanked by 2 *sap* homologs in opposite orientations (*sapA* and *sapA2*) in wildtype strain 23D. Because the 626bp 5' regions are identical, the splice is seamless after the DNA inversion. A 15bp inverted repeat (IR) immediately upstream of the translation initiation codon in each homolog is a putative invertase-recognition site. To define the role of the IR, we mutagenized the IR upstream of *sapA2* to create a *Not*I site. This mutation was introduced to create *C. fetus* strain 23D:ACA2K101N, which has promoterless chloramphenicol- (cm-) and kanamycin- (km-) resistance cassettes inserted at bp127 in *sapA* and *sapA2*, respectively. By alternatively selecting colonies with cm or km-resistance, we could assess the frequency of inversion. We found that mutagenizing the IR did not ablate the inversion. Mapping the unique *Not*I site in relation to the cm or km cassettes in the strains that changed phenotype showed that the splice could occur either in the 66bp upstream or 127bp downstream of the site. By PCR analysis, we also found that the splice could occur in the 425 conserved bp downstream of the cassettes. In total, these data indicate that *C. fetus* can use multiple sites within the 626bp conserved 5' region for its *sap*-related DNA inversion. This appears to be a unique mechanism for DNA inversion.

## The *Campylobacter fetus* SapA secretion signal is located at its C-terminus. SA THOMPSON, KC RAY, MH BEINS, ZC TU and MJ BLASER. Medical College of Georgia, Augusta, GA, and Vanderbilt University, Nashville, TN CP19

*Campylobacter fetus* surface layer proteins (SLPs) are transported to the cell surface by a type I secretion apparatus, which in other bacteria typically recognize C-terminal secretion signals on the secreted protein. To localize and to begin to characterize the secretion signals of *C. fetus* SLPs, we exploited the ability of the cloned *C. fetus* transporter complex (containing SapDEF) to secrete an SLP (SapA) from *E. coli*. We first used PCR to construct a series of SapA derivatives that were deleted at either their N- or C-termini. Wild-type SapA or SapA deletion mutants then were tested for their abilities to be secreted from *E. coli* by the SapDEF transporter. Wild-type SapA (932 amino acids) and N-terminal deletions of 393 and 792 amino acids were detected in *E. coli* culture supernatants, indicating that the N-terminus of SapA was not necessary for its secretion. A 100 amino acid C-terminal peptide was not sufficiently immunogenic to ascertain its secretion proficiency, but a fusion protein containing an immunogenic 187 amino acid N–terminal fragment of SapA and the 100 amino acid C-terminal peptide was secreted. C-terminal deletions of 5 and 10 amino acids remained fully secretion competent; a deletion of 15 amino acids was secreted, but at a lower level than wild-type. Deletions of ≥20 amino acids resulted in no secretion. Therefore, the SapA secretion signal is located in the region between 10 and 100 amino acids from the C-terminus of the protein. This region contains a number of amino acids and predicted secondary structures that are conserved among seven *C. fetus* SLPs and which may be involved in the secretion process.

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## Colonization of non-motile mutants of *Campylobacter jejuni* 81116 in chickens CP20

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Three different non-motile mutants, selected from an insertional mutant library of *Campylobacter jejuni* 81116, were chosen to study their colonization capacity in broilers. These mutants showed a 100 fold reduction in an in vitro invasion assay with INT407 and with chicken macrophage cells (HD11), compared to the wild type strain. Ten day old broilers were orally inoculated with $1.10^5$ bacteria per chicken. On days 2, 4, 7, 9, 11, 14, 21, 28, and 35 after infection, five animals per group were euthanised. Campylobacter CFU's were determined from caecal contents. In the control animals (strain 81116) $1.10^9$ bacteria per gram caecal contents were detected. The BG1 mutant which was a knock-out mutant of the pfla-gene (phenotype: paralysed flagella) was not able to colonize except for one animal per group at a few days with strongly reduced CFU's. Colonization of mutant BG3 (3' part of the flaA gene and the complete flaB gene missing) which showed phenotypically no flagella was delayed and the final level of colonization was about 2log10 lower compared to the wildtype. Mutant BC7 disrupted in the flgK gene (phenotype: stump flagella) showed a diverse colonization pattern. The final level of colonization differed strongly and at each sampling two or three animals were present which were not colonized at all. The highest level of colonization within this group was $3.10^7$/gr. Overall, the colonization declined during the experiment. From this study it becomes clear that non-motile mutants are able to colonize however to a lower level compared to the wild type. The role of motility and the presence of the flagella in colonization will be discussed.

**CP21** Feasability studies on the use of potentially non-pathogenic *Campylobacter* strains as competitive excluders of pathogenic strains in chickens.

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Competitive exclusion (CE) in poultry is a potential strategy for reducing the numbers of pathogenic campylobacters entering the food-chain. The efficacy of normal avian gut flora as CE agents is still debatable. However, the use of campylobacters as homologous CE agents is an alternative. Such strains must be high colonisers, non-pathogenic and stable. Non-pathogenic campylobacters may be either attenuated by genetic modification or naturally occurring strains incapable of causing disease in humans. We have investigated the feasability of using such campylobacters in a chick model of colonisation. In the first study chicks were colonised by a defined superoxide dismutase (SOD) mutant of *C.coli* UA585. The chicks were subsequently challenged with $5x10^2$ -$5x10^5$ cfu of the wild type (WT) parent strain and killed 5 days later. All the birds were still colonised by the $SOD^-$ strain ($10^4$-$10^7$ cfu/g). WT organisms were recovered only from birds challenged with $5x10^4$ (1/10) and $5x10^5$cfu (3/9). In a control group, all birds were maximally colonised ($10^8$ cfu/g) by a dose of $5x10^2$cfu WT. In a second study, chicks were challenged with a chicken strain (CS) with a *fla* type not yet seen in humans and with a human strain (HS) with a *fla* type not seen in chickens. When the strains were given simultaneously at ratios of 1:1, 2:1 and 1:2, only the HS strain was recovered. However, when the CS strain was allowed to colonise for 5 days before challenge with the HS strain, both strains were recovered up to 5 days later but only the CS strain was recovered 7 days later. These results suggest that the first colonising strain can exclude strains encountered at a later date even if these strains have a superior colonisation potential. Thus if non-pathogenic campylobacters can be definitely identified, CE is a viable intervention strategy.

**CP22** PEB3, a Role in the Colonisation of Chicken by *Campylobacter jejuni*.

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Veterinary Laboratories Agency (Weybridge), U.K.

The identification of colonisation factors of *C.jejuni* in poultry could play an important part in the control of the organism in the food chain. PEB3, a 30kDa protein, was one of four *C.jejuni* outer membrane proteins identified by Pei, Ellison and Blaser in 1991. PEB3 is a major antigenic component of *C. jejuni* during chicken colonisation and its expression is upregulated in the avian gut environment. From the genome sequence data PEB3 was found to have significant amino acid homology with AcfC (54%), a putative colonisation determinant *in Vibrio Cholerae* and Anm (53%), an attaching and effacing factor of *E.coli*. It is therefore hypothesised that PEB3 has a virulenvce role possibly in the adherence of *C. jejuni* to intestinal enterocytes and gut colonisation. The surface expression and high antigenicity of PEB3, coupled with the possibility of it being a major pathogenic determinant, makes PEB3 a suitable candidate for subunit vaccine. We have cloned the *C.jejuni peb3* gene from strain 81116 using primers derived from the genome sequence of strain 11168. Sequence comparison of the two genes suggest that *peb3* is highly conserved between the two strains. Primers based on the derived sequence were used to amplify the whole 750bp open reading frame (ORF) by inverse PCR. The ORF was disrupted with the insertion of a kanamycin resistance cassette and the mutation was introduced into the chromosomal copy by homologous recombination. We report on the role of this gene both *in vitro* models of adherence and invasion and a chick oral colonisation model.

NOTES

CP23

Identification and Characterisation of *Campylobacter jejuni* Genes Involved in Host Cell Invasion.

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The mechanism by which *C. jejuni* causes disease is as yet unclear. There is clinical evidence to suggest that host cell invasion is an important part of the infection process. In order to identify bacterial factors required for this invasiveness we used a gentamicin invasion assay to screen a cosmid library of *C. jejuni* strain 81116 for invading clones. The library was prepared in the *E.coli* host HB101 which was non-invasive. A total of forty invasive clones were identified using assays with or without centrifugation. Six clones were invasive in both assays. Two of the highest invading clones (pBT9737 and pBT9724) were selected for further investigation. A random subcloning and sequencing approach with pBT9737 has identifed a large number of genes, some of which may be involved in pathogenesis, however no homology to known invasins has resulted. A number of homologies to genes involved in flagellar biosynthesis and motility were identified. Electron microscopy revealed the presence of type-1-like fimbriae on the surface of all the invasive clones. Mannose sensitive haemagglutination (MSHA) was used to screen the whole cosmid library and only 8% of clones expressed fimbriae, half of which were invasive. Similar fimbriae are expressed by HB101 only following several rounds of serial passage in static broth. These results suggest the presence of a regulator within the cosmid insert which induces fimbrial expression in HB101.

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CP24

The identification of hyperinvasive strains of *Campylobacter jejuni*.

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Both clinical symptoms, such as bloody diarrhoea, the presence of inflammatory cells in stools and occasional bactereamia, and experimental evidence, from *in vivo* and *in vitro* models, suggest that campylobacters can be invasive. However the variability in invasiveness of campylobacter strains, isolated from a number of different but geographically-related environments, has not previously been systematically investigated. The aim of this study was to compare the invasiveness of C. *jejuni* strains isolated from poultry and from the environment surrounding the broiler house, including from wild birds, other domestic animals on site and surface water. Invasiveness was determined using a quantitative gentamicin-protection assay modified to utilise the human foetal intestinal cell line, INT407, in 24-well plates. The results showed that campylobacter strains vary significantly in their invasive potential. Two extremes of invasiveness were identified: a highly invasive type (with approx. 0.05% of the bacteria invading) and a poorly invasive type, (with 0.001%, or less, invading bacteria). Two isolates, one from chicken and another from a puddle, exemplified a extremely hyperinvasive phenotype, which was at least 10 times more invasive than all other strains tested (approx. 0.5% of the bacteria invading). Multiple isolates from the same puddle demonstrated identical phenotype and genotypes. There was no obvious relationship between the invasive phenotype and either *fla*-type or the environmental source of isolates. These results demonstrate that campylobacters show a spectrum of invasiveness, and that some are highly invasive. The relationships between such hyperinvasiveness and host disease presentation has yet to be established.

## CP25 INTERRELATION BETWEEN CAMPYLOBACTER AND SALMONELLA INFECTIONS, ANTIBODIES AGAINTS THESE ORGANISMS AND PATHOLOGICALFINDINGS IN COMMERCIAL LAYERS

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At the end of the production period, 29 layers each from 70 different flocks were examined for the occurrence of campylobacters in their caecal contents. All flocks were found campylobacter positive. Up to 20 individuals of 13 flocks and 21 to 29 birds of 57 flocks were carriers of *Campylobacter* spp. Material of the liver was additionally cultured from birds of 36 flocks. The isolation of the organism from livers was possible only from 1 to 6 birds of 16 flocks. Precipitating antibodies against campylobacters had a positive correlation with antibody titers in the ELISA, and both kinds of campylobacter antibodies correlated with pathological signs of diseases. In contrast to that a negative correlation of antibodies was found between campylobacters and salmonellas and conversely.

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## CP26 COURSE CAMPYLOBACTER INFECTIONS AND ANTIBODIES IN TURKEYS

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Commercial turkey flocks were examined in 2-week intervals culturally for campylobacter infections and serologically for antibodies against campylobacters. The cultural examination of cloacal swabs of living birds and cecal contents at slaughter was positive in 17 of 19 flocks. During the fattening period, there was no specific point time for the first isolation of campylobacters in the flocks. In clinically healthy flocks infected with campylobacters in the first third of fattening period, the antibodies were evident 6 to 10 weeks after the first cultural isolation. Flocks with infection during the central third of the fattening period developed antibodies within 4 to 6 weeks. Flocks infected in the last third showed serological conversion already before or simultaneously with the cultural isolation. Antibodies developed earlier in turkeys diseased with coccidiosis than in turkeys without clinical coccidiosis.

CP27

Cloning and Characterization of a Novel Lipoprotein of *Campylobacter jejuni*

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Analysis of the *Campylobacter jejuni* TGH9011 (ATCC 43431) DNA insert present in recombinant plasmid pHIP-O (E. K. Hani, and V. L. Chan, J. Bacteriol. 177:2396-2402) identified a 1122-bp open reading frame, designated *jlpA*, which encoded a lipoprotein of *C. jejuni*. The predicted gene product of *jlpA* was a 42.6 kDa polypeptide that contained a typical signal peptide and lipoprotein processing site. The presence of a lipid moiety on the JlpA molecule was shown by incorporation of radioactive palmitic acid. A database search found no significant homologies to the deduced JlpA amino acid sequence. PCR and Southern hybridization analyses indicated that *jlpA* was conserved among *C. jejuni* isolates and absent from related *Campylobacter* species. An isogenic *C. jejuni* mutant containing an insertion in *jlpA* exhibited a reduced level of adhesion to HEp2 cells (20.2±2.1% relative to wild-type), implicating possible involvement in cell adhesion. Anti-JlpA antibodies have been generated and are being used in localizing the JlpA proteins within *C. jejuni* cells.

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CP28

Structure and Expression of a Novel Flagellin-like Gene of *Campylobacter jejuni* TGH9011

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We report the identification and characterization of a novel flagellin-like gene *flaC*. The *flaC* gene is predicted to encode a polypeptide (FlaC) of 249 aa, which has 26% identity and 46% similarity to the full length of the flagellin (272 aa) of *Bacillus* sp. C-125. The N-terminal of FlaC (N12-A125) of TGH9011 has 33% identity and 57% similarity to the N-terminal of FlaA of *C. jejuni* NCTC11168, and the C-terminal (T126-Q249) shares 32% identity and 51% similarity. A homolog of the *C. jejuni flaC* gene can be detected in the genomes of *C. coli*, *C. lari* and *C. upsaliensis*. Expression of *flaC* was demonstrated by Northern and Western analyses. Three tandem transcriptional start sites were mapped. Three Fur-box-like sequences were identified that has affinity to purified *C. jejuni* GST-Fur protein suggesting Fur regulation. Furthermore, the level of *flaC* mRNA was reduced in iron-excess conditions indicating iron regulation. A null mutant of *flaC* was constructed and the mutant shows a higher level of motility than wild type parental cells supporting the notion that FlaC is assembled into the flagellar filament.

CP29 **Host PI-3 Kinase-Dependent Pathway is Activated by *Campylobacter jejuni* Secreted Factor.** S. R. RUSCHKOWSKI and B. B. FINLAY. Biotechnology Dept., 237-6174 University Blvd., University of British Columbia, Vancouver, British Columbia, V6T 1Z4 CANADA.

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*Campylobacter jejuni* is the most common bacterial cause of diarrheal disease in humans in North America. However, the pathogenesis of infection is poorly understood and little is known about how *C. jejuni* affects host cell signaling. We have found a secreted factor in the supernatant of *C. jejuni* cultures that activates a PI-3 kinase-dependent pathway. This secreted factor is capable of activating PI-3 kinase in both macrophages and epithelial cells and is inhibited by both wortmannin and LY294002. It is heat-stable and trypsin-insensitive. Cytoskeletal components do not appear to be involved in this process, since cytochalasin D treatment of cells does not reduce this activation. As well, this phenomenon is not mediated by *Campylobacter* LPS. Activation of this PI-3 kinase-dependent pathway is known to inhibit apoptosis. Since, *C. jejuni* is able to survive and persist within monolayers of J774A.1 and Henle cells for at least 24 hrs, activation of this pathway may enable bacteria to replicate within the host organism before cell death occurs. This secreted factor may be necessary in the pathogenesis of *C. jejuni.*

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CP30 Differences in Virulence between *Campylobacter jejuni* 81-176 and NCTC 11168. D. BACON, C. P. EWING, D. H. BURR, D. M. ROLLINS and P. GUERRY. Naval Medical Research Center, Bethesda, MD and Food and Drug Administration, Laurel, MD

Strain 81-176 (O:23, 36) is one of the best characterized strains of *C. jejuni* and has been shown to cause disease in two human feeding studies. Strain NCTC11168 (O:2) is a clinical isolate whose genome has been sequenced by the Sanger Centre. The relative virulence of *C. jejuni* 81-176 and NCTC11168 was compared in both in vitro and in vivo assays. NCTC 11168 adhered to INT407 cells at approximately 11% the level of 81-176 and invaded INT407 cells at <1% the level of 81-176. Ferrets were fed two doses of each strain. The high dose was approximately $10^{10}$ organisms and the low dose was approximately $10^{9}$ organisms. When the high dose of 81-176 were fed to ferrets, 100% of the animals (8/8) developed diarrhea; at the low dose 50% (4/8) of the animals developed diarrhea. At the high dose of NCTC11168 only 11% of the ferrets (1/9) developed diarrhea, and at the low dose no animals showed any symptoms (0/8). We have identified a gene in 81-176, which is not found in the genome of NCTC11168, that encodes a protein with significant homology to a protein found in type IV secretion systems of other pathogens. Mutation of this gene in 81-176 resulted in a reduction of adherence and invasion of INT407 to approximately 10% of that seen in wildtype. When this mutant was used to infect ferrets at the high dose only 50% of the animals (4/8) developed diarrhea, and at the low dose no animals became ill (0/8). Collectively, these data suggest that there are different mechanisms of virulence among strains of *C. jejuni*.

CP31

Colonic lymphoglandular complexes are a major site for IgA induction in pigs with invasive *Campylobacter jejuni* L.S. MANSFIELD[1], S.R. ABNER[1], D.T. GAUTHIER[1] and J.F. URBAN[2] [1]Michigan State University,East Lansing, MI,[2]USDA,Beltsville, MD.

Gnotobiotic pigs infected with both *T. suis* (250 eggs/kg) and *C. jejuni* ($10^6$ CFU of log phase bacteria, ATTC strain 33292 from a human with enteritis) had mucohemorrhagic diarrhea and severe pathology in the colon at 27 days after infection. Pigs with *Trichuris*, or *C. jejuni* alone, or no pathogens, had no disease and no pathology. Pigs with both *T. suis* and *C. jejuni* had significantly enlarged lymphoglandular complexes (LGC) in the distal colon (2.4 + 0.4 mm in size) compared to uninfected controls (0.75 + 0.28 mm), pigs with *C. jejuni* alone (1.3 + 0.6 mm) and pigs with *T.suis* alone (0.8 + 0.5 mm)($P<0.05$,Kruskal-Wallis rank sums test). Pigs given *C. jejuni* alone had developed germinal centers in the LGCs without a statistically significant increase in follicle size. Histopathology with H&E and Warthin-Starry staining, and immunohistochemistry revealed that pigs with dual infections had greater numbers of *C. jejuni*. These bacteria were found at the base of crypts in the proximal colon, and within goblet cells and epithelial cells in the mucosa. Also, submucosal LGC were invaded by *C. jejuni* in these pigs; bacteria were found in the follicle-associated epithelium and within macrophages of the lymphoid follicle. Pigs given *C. jejuni* alone had fewer numbers of *C. jejuni* than dual infected pigs and bacteria were found extra-cellularly in mucus and in goblet cells in the superficial mucosa. The cell types in the LGCs from all groups were analyzed by immunohistochemistry using monoclonal antibodies for cell surface markers IgM, IgG, IgA, CD4, CD8, MHC Class II, and macrophage marker SWC3a. The cellular composition of the lymphoid tissue within the LGCs was the same in all groups, but the number of cells was increased depending on infection status. Low numbers of $CD4^+$ and $CD8^+$ T cells in mantle areas, and significant populations of $IgM^+$ B cells were found in the LGC follicle in all groups. Macrophages were located in the periphery of germinal centers. $IgA^+$ B cells were seen in high numbers in the lamina propria and in the germinal centers of LGCs of groups receiving *C. jejuni*. We conclude that LGCs are secondary lymphoid organs invaded by *C. jejuni*.

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## Mechanism of Invasion of INT-407 Cells by *Campylobacter jejuni*.

CP32

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*Campylobacter jejuni* is a major cause of gastroenteritis, but the mechanism of infection is poorly understood. It has been suggested that adherence to and invasion of intestinal epithelial cells are important virulence factors for *C. jejuni*. In the present study, 32 *C. jejuni* strains including clinical and healthy animal isolates were compared in invasion assays. On the basis of the source of isolation, there was no significant difference in adherence to the cells but the invasion ability of *C. jejuni* varied considerably.

Invasion of INT-407 cells by *C. jejuni* was inhibited 50 to 78% by microfilament depolymerization for five out of 10 strains and was inhibited by both microfilament and microtubule depolymerization for four out of 10 strains . Inhibition of coated-pit formation, as well as bacterial endocytosis and endosome acidification also reduced invasion of INT-407 cells by *C. jejuni* by 62 to 86% and 72 to 93%, repectively. Furthermore protein kinases, but not phosphoinositide 3-kinase or protein kinase C, showed 60 to 80% inhibition of invasion of INT-407 cells by *C. jejuni* . These results suggest that the internalization mechanism triggered by *C. jejuni* is strikingly different from the microfilament-dependent invasion mechanism exhibited by other enteric pathogenic bacteria.

**CP33** Biological properties of cytolethal distending toxin T. E. HICKEY, A. L. MCVEIGH, S.A. CARROLL, D. A. SCOTT, A. L. BOURGEOIS, and P. GUERRY Naval Medical Research Center, Bethesda MD

Cytolethal distending toxin (CDT) of *C. jejuni* 81-176 has been shown to block eukaryotic cell division in the $GM_2$/M phase of the cell cycle. In this report we examine properties of CDT expression, and demonstrate that functional CDT molecules mediate the release of IL-8, a potent pro-inflammatory cytokine, from intestinal epithelial cells. CDT expression was studied with non-polar insertional mutants in each of the subunit genes; *C. jejuni* CDT is encoded by 3 subunit genes that are co-transcribed. We found gene product expression to be co-dependent, and each product to localize to bacterial membranes. The CdtA subunit was shown to be a lipoprotein through protein sequence analysis and confirmed through palmitic acid labeling. CdtA is required for localization of CdtB and CdtC; none of the CDT subunits were expressed in *cdtA* mutants, and neither CdtB nor CdtC were observed in *cdtB* or *cdtC* mutants. We have previously shown that live Campylobacters can induce the release of IL-8 from intestinal epithelial monolayer cells. We now demonstrate that membrane fractions containing CDT are also capable of mediating IL-8 secretion in the absence of viable bacteria. *C.jejuni* 81-176 membrane fractions ($CDT^+$) were reactive, while*C. coli* ($CDT^-$) membranes were not. Additionally, membranes from a *C. jejuni cdtA* mutant (DS104) were non-reactive in both CDT and IL-8 assays, but both reactivities were restored when complemented *in trans* with pRAM33 which contained the wildtype CDT operon. *C. coli* 12498 membranes are $CDT^-$ as assessed by bioassays and immunoblots, and were also inactive in the IL-8 assay until complemented *in trans* with pRAM33. The pro-inflammatory properties of CDT is not restricted to *Campylobacter spp.* Membrane fractions from $DH5_\alpha$ transformed with plasmid pDS7.96, containing the wild type *E. coli* CDT operon, mediated strong IL-8 responses. These data suggest that CDT may contribute to the host inflammatory response and ensuing pathology during enteric infections.

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**CP34** **A mouse model for *Campylobacter jejuni* pathogenesis**

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The knowledge on the virulence mechanisms for campylobacters is very limited at the moment. An animal model of *Campylobacter* pathogenesis would be a valuable tool in the differentiation between more or less virulent *Campylobacter* strains and to identify and characterize possible virulence factors. Most of the rather few published *in vivo* models of *Campylobacter* pathogenesis are either only colonization models (the animals show no clinical symptoms), use unpractical laboratory animals (e.g. ferrets, neonatal mice), or the isolates have do undergo several *in vivo* passages to obtain measurable effect in the model. The purpose of this study was to develop a simple in vivo model with clear and measurable effects and with a presumable close simulation of the pathogenesis of *C. jejuni* in humans, i.e. diarrhea, weight loss.

Mice (adult Balb/C mice) were challenged i.p. with a single dose of live *C. jejuni* cells supplemented with iron dextran. From the time of challenge until four days later, the mice were observed for diarrhea, other illness symptoms/death, the body weight was measured, and the concentration of the challenge strain excreted in feces was determined. On day four, the relative weight of the spleen and liver was measured along with the concentration of *C. jejuni* cells in small intestine, liver and spleen.

A clear dose-response effect on several parameters, e.g. symptoms of diarrhea and sickness, weight loss, enlarged spleen and death was seen for a *C. jejuni* strain tested at four dose levels. This strain had been implicated in a human outbreak of campylobacteriosis. When mice were challenged with the same concentrations of heat-killed cells a significantly weaker response was seen, i.e. in the group with the highest dose a temporary weight loss and slightly enlarged spleen was seen, but no animals died or showed clear clinical symptoms.

The model was used for testing of other *C. jejuni* strains using a single high dose. Different strains clearly demonstrated different degrees of effect in the model.

CP35

Characterisation of the Cytolethal Distending Toxin of *Campylobacter upsaliensis*. MOONEY A, CURRAN T, CLYNE M, DOHERTY D, BOURKE B, Dept Paediatrics, Univ College Dublin, Children's Research Inst, Our Lady's Hospital, Dublin.

BACKGROUND: *Campylobacter upsaliensis* is an emerging human enteropathogen recently also associated with Guillain Barre syndrome. However, little is known about the pathogenesis of *C. upsaliensis* infection.
AIM: To characterise the cytolethal distending toxin (CDT) of *C. upsaliensis*.
METHODS AND RESULTS: (1) CDT activity of the *C. upsaliensis* type strain (ATCC 43954) and 4 clinical isolates (Laboratory Centre for Disease Control, Ottawa, ON) was assayed using a standard tissue culture model. Hela cells exposed to *C. upsaliensis* lysates demonstrated progressive distension culminating in cell death over 5 days. Intact bacteria and bacterial supernatents also produced weak cytolethal distending phenotype. Immunoflourescent microscopy demonstrated nuclear fragmentation among treated tissue culture cells. Bacterial lysate treated Hela cells subjected to FacsScan analysis using CFDA SE as a cell tracer demonstrated cell division arrest. (2) Degenerate oligonucleotide primers and inverse PCR were used to generate *cdt*B gene sequence from *C. upsaliensis* ATCC 43954. Initial sequence analysis indicates both conserved and non-conserved regions of *C. upsaliensis cdt* compared with the *C. jejuni* homologue. Genomic DNA isolated from 25 clinical isolates of *C. upsaliensis* was subjected to Southern analysis using a region of *cdt*B. All 25 isolates contain sequences with homology to the *cdt*B gene.
CONCLUSIONS: CDT is the first virulence factor described in *C. upsaliensis*. *C. upsaliensis* CDT produces effects on tissue culture cells comparable to CDT from other species and appears to be common to most *C. upsaliensis* strains.

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CP36

In vitro and in vivo Assays to Measure the Virulence of Campylobacter jejuni D E STEWART-TULL*, J G COOTE*, H THOMPSON§, A C WARDLAW*, R OWEN§§, F BOLTON$, A CANDLISH* and D CANDLISH*.
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Forty genotyped and serotyped strains of C.jejuni were obtained from three public health centres in the UK. These were given code numbers so that a blinded study could be done. Initially, the strains were subcultured and a master stock of freeze-dried ampoules was prepared. No strain ampoule was used beyond 5 sub-cultures before a new one was opened. The strains were biochemically analysed to confirm C.jejuni before testing for their haemolytic activity. Subsequently, each strain was tested for a) 7-day-old mouse toxicity test, b) cytotoxicity against various cell lines, c) adhesion and invasion of the cell lines, d) 11-day-old chick embryo assay as a measure of lethality e) ileal loop assay in mice and chicks as a measure of intestinal fluid accumulation and cytotoxicity and f) histopathology of ileal sections. An assessment of the results was undertaken once the code was broken; there were 24 human strains, 5 bovine strains, 8 chicken strains , 1 ovine strain, 1 strain from milk and 1 unknown source.
There was a wide variation in the activity of all strains in these assays. Test a) was stopped because the results were non-discriminatory. The results of the other assays were graded to allow statistical analyses of the data. In the cytotoxicity assay some strains were active against HeLa and Vero cells but inactive against CaCO-2 cells and vice versa. A relationship between a positive result in this test and the results of the chick embryo and ileal loop assay was apparent. With the chick embryo assay a two dose system was used, at the high dose it was concluded that a critical level of endotoxin in the egg could be responsible for the death of the embryo, but with the low dose of organisms the embryo was killed before this level was reached; this indicated that another factor was involved. There was also some correlation between results in the egg embryo assay and the extent of the pathology in histological sections of the ileal loop.

**CP37** Characterization of the secretion of the Cia proteins from *Campylobacter jejuni*. VANESSA RIVERA-AMILL AND MICHAEL E. KONKEL. Department of Microbiology, Washington State University, Pullman, WA 99164-4233

NOTES

*Campylobacter jejuni* secrete at least eight proteins termed Cia for *Campylobacter* invasion antigens, which are required for *C. jejuni* entry into host cells, upon incubation with INT 407 cells or INT 407-cell conditioned medium. The purpose of this study was to identify a signal capable of stimulating the synthesis and secretion of the Cia proteins. Initial experiments revealed that the Cia proteins were secreted upon cultivation of *C. jejuni* in fetal bovine serum (FBS)-supplemented medium. The stimulatory molecule was not unique to FBS as sera from other sources including human, pig, sheep, goat rabbit, mouse and chicken also induced the synthesis and release of the Cia proteins. Confocal microscopy examination using an antibody directed against CiaB, one of the secreted proteins, revealed that *C. jejuni* readily stained with the anti-CiaB antibody when cultured in FBS-supplemented medium. *C. jejuni* were not stained when incubated in medium without FBS. Contact of *C. jejuni* with INT 407 cells cultured in serum-free medium also stimulated the synthesis and secretion of the Cia proteins, but secretion did not occur in the presence of glutaraldehyde-fixed cells. Additional experiments revealed that the Cia proteins are synthesized and secreted in the presence of a stimulatory signal, and that the synthesis and secretion processes can be uncoupled. Temporal kinetic studies revealed a direct correlation between *C. jejuni* entry and secretion of the Cia proteins. The experimental data indicate that secretion of the Cia proteins is stimulated by incubating *C. jejuni* in medium supplemented with serum or in the presence of intestinal cells.

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**CP38** Confocal microscopic examination of the adherence of a *Campylobacter jejuni* parental strain and a *cadF* mutant to INT 407 epithelial cells. STEVEN G. GARVIS AND MICHAEL E. KONKEL: School of Molecular Biosciences, Washington State University, Pullman WA 99164-4233

Eukaryotic cells possess a variety of extracellular matrix molecules, integrins, and associated cytoskeletal elements for adherence to the substratum, movement, and cellular signaling. An emerging theme among pathogenic microorganisms is their ability to utilize these cellular molecules during the infectious process. We recently identified a 37 kDa outer membrane protein, termed CadF, from *Campylobacter jejuni* that mediates the organism's binding to the extracellular matrix component fibronectin. To further characterize the interactions occurring between *C. jejuni* and host epithelial cells, in vitro binding assays were performed in conjunction with confocal microscopy studies. In vitro binding assays revealed a significant reduction in the binding of the *C. jejuni cadF* mutant to INT 407 cells when compared to the *C. jejuni* parental strain. Confocal microscopy examination of INT 407 cells infected with the *C. jejuni* parental and the *cadF* mutant strains revealed marked differences in binding patterns. The *C. jejuni* parental strain, in contrast to the *C. jejuni cadF* mutant, bound to cell-associated fibronectin. Furthermore, colocalization was observed between the *C. jejuni* parental strain and $\alpha_5$ integrin subunit. These initial studies suggest that fibronectin may serve as a bridge between *C. jejuni* and host epithelial cells, facilitating the adherence required for the infectious process.

CP39

## The *galE* gene of *Campylobacter jejuni* is involved in lipopolysaccharide biosynthesis.

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Lipopolysaccharide (LPS) is one of the main virulence factors of Gram-negative bacteria. The LPS from *Campylobacter* spp. has endotoxic properties and has been shown to play a role in adhesion. We have cloned a gene cluster (*wla*) which is involved in the synthesis of the *C. jejuni* 81116 LPS molecule. Sequence alignment of the first gene in the *wla* cluster indicated similarity with *galE* genes of *S. typhimurium*, *H. influenzae*, *E. coli* and *N. meningitidis*. These genes encode a UDP-glucose 4-epimerase, which catalyzes the interconversion of UDP-galactose and UDP-glucose. Sequence analysis of the *C. jejuni galE* gene revealed serine 124, tyrosine 149, lysine 153 and a nicotinamide adenosine dinucleotide-binding domain, which are characteristic for epimerases. A *Salmonella galE*⁻ mutant was transformed with a plasmid construct containing the *galE* gene from *C. jejuni*. The LPS analysis of a wild-type, *galE*⁻ and complemented *galE*⁻ *Salmonella* strain showed the *galE* gene from *C. jejuni* could restore the smooth LPS pattern as shown in SDS-polyacrylamide gels. A UDP-glucose 4-epimerase assay was used to demonstrate that the *galE* gene from *C. jejuni* encodes for this epimerase. A *galE*⁻ *C. jejuni* mutant was constructed by deleting part of the *galE* gene and inserting a kanamycin resistance gene. The Lipid A-core complex from this mutant had a reduced molecular weight and did not react with antiserum raised against LPS of the parental strain. The results presented here are consistent with an essential role for the *galE* gene in the synthesis of *C. jejuni* lipopolysaccharides.

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CP40

## The isolation and characterization of a second gene cluster involved in lipopolysaccharide synthesis in *Campylobacter jejuni*.

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We have previously isolated a *C. jejuni* strain 81116 gene cluster that is involved in LPS synthesis. Here we report on the isolation of a second gene cluster that is also involved in LPS synthesis. A DNA region of 5 kb was isolated and sequence analysis revealed that it contained five open reading frames. The first gene, *waaC*, is similar to heptosyltransferase-I, an enzyme attaching the first heptose residue to the Kdo molecule of the inner core. The second gene, *wlaNA*, showed similarity to lauroyl acyltransferase, which is involved in lipid A synthesis. The *wlaNB* gene was similar to the *igtF* gene from *Neisseria meningitidis*, encoding a glucosyltransferase, which attaches the first glucose residue to the first heptose residue of the LPS. The genes *wlaNC* and *wlaND* showed similarities to genes involved in rhamnose synthesis. To further analyze the *waaC* gene a *Salmonella typhimurium waaC*-negative mutant was transformed with the *C. jejuni waaC* gene. Analysis of the LPS from the wild type, mutant and complemented mutant of *S. thyphimurium* showed that the *C. jejuni waaC* could restore the LPS synthesis to the wild type form. We were not able to inactivate the *waaC* gene of *C. jejuni*, indicating that this gene is essential for this bacterium. The presence of the genes in this cluster in 20 *C. jejuni* Penner serotype strains was studied by PCR and Southern blot. The *waaC* and *wlaNA* genes were found in all strains, while the *wlaNB*, *wlaNC* and *wlaND* genes were absent in most of the strains. Thus this second LPS gene cluster is variable in gene content between *C. jejuni* strains.

CP41 **Lipopolysaccharide is a virulence factor in *Campylobacter jejuni*.**

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Dept. Applied Biology and Biotechnology, RMIT-University, Melbourne, Australia. [1]Veterinary Laboratories Agency (Weybridge), UK.

Lipopolysaccharide (LPS) molecules are a major constituent of the bacterial cell wall. They are found in all Gram-negative bacteria and are one of the main virulence factors. *C. jejuni* strains express a low molecular weight LPS molecule and in addition some strains also express O-antigen-like carbohydrate repeats. The LPS structure of *C. jejuni* is highly variable. Surprising is the presence of N-acetylneuraminic acid (NeuNAc), a molecule usually not found in prokaryotes. These NeuNAc residues when attached by 2-3 linkages to β-D-Gal resemble gangliosides. This molecular mimicry is thought to play a role in the neuropathological autoimmune disease the Guillain-Barré syndrome. We have previously constructed a LPS mutant in *C. jejuni* 81116 by inactivating the *galE* gene. The LPS from this mutant is truncated compared to the wildtype LPS and does not react with the strain-specific antiserum. To investigate the role of LPS in disease several virulence indicators of this mutant were studied. The LPS mutant showed a reduction in its ability to adhere to and invade INT407 cells compared to the parent strain, 1% and 5% respectively. However, it was still able to colonize chickens to the same level as the wildtype strain. The serum resistance of this mutant seems to be slightly increased, whereas no difference was seen in hemolytic activity compared to the parent strain. The ability of the mutant to take up DNA and integrate it in its genome was reduced 100 fold compared to the wildtype. These results show that the LPS of *Campylobacter jejuni* is an important virulence factor.

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CP42 The Influence of various Microorganisms on the Growth of *Helicobacter pylori* and *Campylobacter jejuni/coli*

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According to the observation that *H. pylori* (H.p.) can be inhibited by certain types of bacteria, as described in other studies, the influence of 31 different species of facultative and obligate anaerobes on the growth of H.p. (n= 31) and *C. jejuni/coli* (C.j./c.) (n= 38) clinical isolates was examined. The inhibitory effects were determined by the "cross-streak" method in two ways: A suspension of the effector organisms was applied to a blood agar plate as a 0.5 mm wide streak and a) when dried off suspensions of H.p. and C.j./c. ($10^5$-$10^6$ cfu/ml) respectively were applied at right angles, or b) after incubation at 37°C for 48 h the microorganisms were killed by exposure to chloroform vapour before applying *Campylobacter* and H.p. strains; growth was recorded after 48 h and 4 days of microaerophilic incubation respectively. All the *Campylobacter* strains were inhibited by *S. pneumoniae*, which had no effect on H.p., further *P. aeruginosa, M. morganii, S. aureus, F. nucleatum and C. difficile* showed an influence on the growth. A growth inhibition of most strains of H.p. was obtained by *S. epidermidis, S. maltophilia, P. aeruginosa, B. fragilis, S. aureus, and M. morganii.* The antibacterial activity of the supernatants of those microorganisms with an inhibitory effect were tested using the agar-diffusion and the macro-dilution test; the detection of bacterial acid products was done using the HPLC-method.
**Conclusion:** The inhibitory activity of effector bacteria varies dependent on species and strains of C.j./c. and H.p. The inhibitory effect could be partly due to the acids (lactic, fumaric, succinic) produced. The pathogenesis of infection with H.p. and C.j./c. might be influenced by the present bacterial flora. The difficulty of cultivating *H. pylori* from faeces could be related to antagonistic bacterial interaction.

## Adhesion, Invasion and Survival Of *Campylobacter hyoilei* in He-La and Int-407 Cells

CP43

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Adhesion and invasion properties of enteric pathogens of *Campylobacter* species to eukaryotic cells in tissue culture are often correlated to their pathogenicity. In this study five strains of *C.hyoilei* isolated from pigs were tested for their ability to adhere and invade HeLa and INT-407 tissue culture cell lines. Their ability to survive within the macrophage cell line J774 was also investigated. The study has shown that the *C.hyoilei* strains adhere and invade human tissue culture cell lines HeLa and INT-407. The variation of adherence and invasion to both cell lines were observed among *C.hyoilei* strains. The percent adhesion of *C.hyoilei* strains to HeLa and INT-407 cells ranged from 0.033% to 0.139%, and 0.034% to 0.14% respectively. Similarly, the percent invasion index among *C.hyoilei* strains ranged from 1.21% to 5.8% for HeLa cells and 2.2% to 6.4% for INT-407 cells. Reference *C.hyoilei* strain 32A showed the highest invasion index. The survival capacity and the multiplication rate of *C.hyoilei* strains within the macrophage cell line J774 was determined by the gentamicin protection assay and revealed that *C.hyoilei* strains did not multiply within macrophages. However, the number of viable cells did not drop significantly after 6 hours and viable bacteria were found within J774 cells after 24 hours. Reference *C.hyoilei* strain 32A showed reduction of surviving cells in the J774 cells from $10^5$ to $10^4$ . Similar results were also obtained for *C.jejuni* 81116, which was used in this study for comparison. These findings indicate that *C.hyoilei* strains can adhere and invade tissue culture cells, but their multiplication and survival capacity in tissue varies considerably.

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Stool Levels of Cytokines and Bacteria-Specific Antibodies in Human Experimental and Naturally-Acquired *Campylobacter jejuni* Infections

CV1

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Cytokines produced in response to infection not only regulate the immune response but also contribute to the pathology of disease process. We studied the role of mucosally produced inflammatory and regulatory cytokines in experimental and naturally-acquired *C. jejuni* (*Cj*) infections. Stool were collected from 28 volunteers before and 3-4 days after feeding $10^5$-$10^9$ cfu of *Cj* in bicarbonate buffer. Stools were also obtained from 23 volunteers experiencing episodes of acute diarrheal disease while in Thailand for a military exercise and from 48 asymptomatic individuals also participating in the same training. Among the 23 diarrheal cases, 10 were shown to be *Cj* infections by culture and the remaining were due to non-typhoidal *Salmonella* infection. Stools were collected from acute cases at 1.5-2 days post-onset of their illness, and stools from asymptomatic controls were collected over the course of the 3 week exercise. *Cj*-specific IgA and IL-1, IL-8, TNF-α, and IFN-γ levels were determined in stool extracts by ELISA.

Controls (asymptomatic and before experimental infection) showed undetectable to low levels (164 pg/ml) of study cytokines in their stool, whereas, 70-100 % of volunteers experimentally infected with *Cj* showed substantial up-regulation of IL-1 (590 pg/ml), IL-8 (691 pg/ml), and TNF-α (480 pg/ml). Natural infection resulted in similar levels of TNF-α (638 pg/ml), a 10-fold higher IL-1 (5,729 pg/ml), and 5 times lower IL-8 (135 pg/ml). In contrast, *Salmonella* infection induced significantly lower levels of all cytokines. Over 55% of the experimentally infected volunteers exhibited IFN-γ induction, whereas, after natural infection only 20% had detectable levels of IFN-γ in their stools. Similarly, bacteria-specific sIgA was detected in all experimentally infected individuals, but only in 50% of those individuals with community acquired illness. Although cytokines response profiles in stools following natural and experimental *Cj* infections tended to differ, they both were distinct from other enteric infections.

CV2

Duration of Protection in Humans following Experimental Infection with *Campylobacter jejuni*

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Healthy seronegative adult volunteers (n = 36) were challenged with Cj ($10^9$ CFU) in bicarbonate buffer and monitored in an inpatient setting for 10 days. From this initial infection (IT) group, subsets of volunteers received either short-term (ST) (n = 8 at 28-49 days) or long-term (LT) (n = 7 at 1 year) rechallenge with the same strain. All volunteers in the IT and LT groups were colonized compared to 75% in the ST group. Maximal fecal colony counts for Cj were comparable in the IT and LT groups (~$10^8$ CFU/gram), but there was a 3 log lower median level in the ST group. Diarrhea attack rates were 83% in IT, 57% in LT and none in ST. 1-yr veterans developing illness demonstrated a less severe illness than the IT group as evidenced by less diarrhea (total volume – 1.1 vs. 1.7 L; stool number – 7 vs. 13), no grossly bloody stools vs. 25% in controls, less fever (14% vs. 44%), and increased tendency to resolve symptoms prior to initiating antibiotic therapy.

Immune responses to Cj antigens were measured in stool (sIgA), plasma and IgA-secreting cells (ASC) present in PBMC. Peak ASC levels were highest amongst the IT group (300-400 range) followed by LT (100-250 range) and ST groups (< 10). sIgA and serologic titers were higher at baseline for both veteran groups as compared to the IT group. Fecal IgA responses were 70-100% in all groups. Serologic responses (median fold-rise) tended to be higher in IT (4-8) than ST (1) or LT (1-8) groups. In summary, complete protection from illness upon re-exposure to a homologous Cj strain was observed to occur 1 month following initial infection but partially waned 1 year later.

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CV3

**Preparation and Quality Control of Live *Campylobacter jejuni* for Experimental Oral Challenge of Human Volunteers**

**D.M. ROLLINS, L.H. LEE, S.W. Walz and A.L. Bourgeois**
**Naval Medical Research Center, Forest Glen, MD**

*Campylobacter jejuni*, strain 81176 (Black, et al., J.I.D., 1988) was grown on Mueller-Hinton soft agar (semi-solid motility agar) (MHSA) and highly motile cells from the periphery of the zone of motility were sub-cultured, expanded and quickly frozen. Under GMP conditions, this NMRI Master Seed Lot was scaled up twice in fermenters and Production Seed (Lot#0043 of PS81176) was prepared. Extensive characterization of the biochemical attributes and growth characterisitics of PS81176 were performed. Growth kinetics were established to optimize oral delivery of a known number of challenge organisms using linear regressions of optical density at 625nm (OD) to predict viable cell numbers. The protocol necessitated delivery of a low ($10^5$), mid ($10^7$), and/or high ($10^9$) bacterial dose in a 1.3% bicarbonate solution. It was determined that at both low and high dose levels, PS81176 was extremely sensitive to suspension in bicarbonate (~.16 log/h die-off at low dose), but stable in PBS. Thus, the challenge organisms were not added to the bicarbonate solution until immediately before ingestion. The final SOP for the human challenge preparation was modified from Black et al. (1988) Extensive quality controls and recordkeeping were performed throughout the SOP. Briefly, Day 1: sBAP inoculated from frozen PS81176; Day 2: After 22-24h, sBAP growth resuspended in thioglycollate (OD=.140) and MHSA inoculated; Day 3: After 20-24h, MHSA growth from periphery of motility zone resuspended and brucella agar inoculated; Day 4: After 18-22h, growth was harvested and suspended (OD=.200) in PBS for oral delivery.

NOTES

CV4

Salivary and serum IgG, but not IgA, antibody levels in patients with a previous *Campylobacter* infection remain elevated over time. S.A.CAWTHRAW AND D.G.NEWELL. Veterinary Laboratories Agency (Weybridge), UK

A non-invasive test to detect a host's previous exposure and immune status would be valuable for epidemiological studies on *Campylobacter* infections. To investigate the kinetics of mucosal antibody responses to *Campylobacter*, multiple whole saliva samples were taken from 60 patients 1-12 months after laboratory-confirmed diagnosis. Sera were also taken at the time of the first and last saliva samples. Samples were taken from a control population with no recent history of diarrhoea. Antibody levels were determined by ELISA, using acid-extracts of *C.jejuni* strain 81116 (AE), an aflagellate mutant of 81116 (R2-AE) and a whole-cell sonicate of R2. The results showed no significant differences between anti-AE/R2-AE IgA levels in the first and last saliva samples from patients, or between patient samples and controls. Anti-AE/R2-AE IgG levels were significantly higher ($p<0.001$) in the initial patient sera than in control sera, but in the second sera the difference was only significant against 81116 AE ($p=0.005$). Salivary IgA and IgG antibodies against R2 sonicate in the first patient samples were significantly higher than in controls ($p=0.029$ and $p<0.001$ respectively). However, only IgG levels remained significantly higher ($p=0.002$) by the time the last saliva samples were taken. A similar pattern was seen in circulating IgA and IgG antibodies against R2 sonicate. The results indicate IgG antibodies, detectable in both serum and saliva, directed against flagellin and other antigens not present in AE, are indicative of previous exposure to *Campylobacter*.

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CV5

The ovine immunological response to the S-layer protein during *C.fetus fetus* infections. R.GROGONO-THOMAS[1], J.DWORKIN[2], M.J.BLASER[2]. M.AHMADI[3] D.G.NEWELL[1,4] 1) Royal Veterinary College. London, U.K. 2) Vanderbilt University, Nashville, TN. USA. 3) University of Tehran, Iran. 4) Veterinary Laboratories Agency (Weybridge). U.K.

A water extract of *C.fetus fetus* primarily comprising S-layer protein (SapA) was used to develop an antibody-capture ELISA to assay IgA, IgG1, IgG2, and IgM antibodies in ovine serum, milk, bile, mucosal washings and faeces. The ELISA confirmed previous results that infected sheep elicit a substantial immune response directed against the SapA protein of *C.fetus fetus*. Antibodies of all isotypes were detectable during experimental and natural infections and up to10 months after abortion. The ability of SapA to induce protective immunity against abortion was investigated. All 5 ewes, which were inoculated with live wild type *C.fetus* strain, 23D, prior to mating produced live lambs following challenge with the homologous strain at day 105 of gestation. In contrast 20% of ewes, inoculated with either a SapA-negative mutant (1/5), or broth alone (1/5) and then subsequently challenged with strain 23D, aborted. Preliminary evidence was also obtained that the response was cross-protective and independent of the SapA protein homolog expressed. Therefore protective immune responses are probably directed against conserved regions of the SapA proteins. The amino acid sequences of three SapA protein homologs (SapA, A1, A2) were compared. A major conserved region was identified (the first 184 amino acids of the N-terminus) which was partly epitope mapped using 17 synthetic peptides (20 mers overlapping by 10 amino acids), produced by Fmoc technology. Serum antibodies from experimentally or naturally infected sheep consistently bound to four synthetic peptides (9, 10, 14 and 15). In contrast, polyclonal rabbit antisera directed against purified SapA bound exclusively to peptide 10. None of the anti-SapA mabs tested bound to any of the peptides. The epitopes identified may be potentially useful as a synthetic subunit vaccines.

**CV6** Systemic Immune and Immune Response-associated Changes Following Naturally Acquired *Campylobacter jejuni* Infection.

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A unique opportunity to define the human immune response to *C. jejuni* resulted from the rapid detection of infection in a volunteer for whom archives of sera and mononuclear cells were available. Stool cultures made on day 3 of clinical disease yielded a *C. jejuni* that could not be typed by Lior serotyping system. Serum collected before infection had low or negative levels of *C. jejuni* specific antibodies detected by ELISA, and were negative for TNF alpha, IL-1 beta IL-8, IL-6, interferon gamma and C reactive protein. Western blots showed recognition of 2 (by IgA) or 7 (IgG) *C. jejuni* proteins. Mononuclear cells showed no antibody secreting cells specific for *C. jejuni* and no enhanced lymphocyte replication response to *C. jejuni* antigen, and normal responses to mitogens. Samples collected during or after infection showed vigorous, rapid and sustained responses to *C. jejuni*. Increases in serum antibodies were found by day 3 of illness, peaked at day 10 (IgM or IgA) or 34 (IgG) and persisted through at least 126 days. With the exception of TNF alpha, marked increases in cytokines and CRP were found with IL-8 and CRP having maximum response at day 3 and the others at day 7. *C. jejuni* specific ASC were detected at high levels at days 3 and 10 and returned to undetectable by day 34. Lymphocyte replication response to *C. jejuni* antigen was positive by day 3 and very strongly positive at 120 days. Western blots showed a vigorous response to *C. jejuni*. For all outcomes immune response to the causative *C. jejuni* were compared to those detected with reference strain 81-176. The causative strain was preferentially recognized. *C. jejuni* infection associated with a marked suppression of mitogen driven lymphocyte proliferation. This is the first detailed analysis of human immune response to naturally acquired *C. jejuni* infection.

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**CV7** Distinct Functional Alleles of Sialic Acid Biosynthetic Genes in *Campylobacter* spp. P. GUERRY, C. P. EWING, D. DAINES, R. P. SILVER, and T. J. TRUST. Naval Medical Research Center, Bethesda, MD, University of Rochester, Rochester, NY, and University of Victoria, Victoria, BC.

The flagellins of *Campylobacter* spp. are posttranslationally modified by glycosyl moieties, which include sialic acid. We previously described two genes required for the biosynthesis of the flagellar posttranslational modification (ptm) of *C. coli* VC167. One of these genes encoded a CMP-NeuNAc synthetase which has significant homology to the *neuA* gene product of *E. coli* K1, required for synthesis of the polysialic acid capsule of this neuropathogen. We have now identified two additional genes in VC167 which encode homologs of the *neuB* and *neuC* genes of *E. coli* K1. Mutation of either of these genes results in a change in the apparent $M_r$ of the flagellin on SDS-PAGE gels, a loss of reactivity with an antiserum specific for the ptm, and a change in the isoelectric focussing pattern of flagellin. All of these phenotypes can be restored by complementation of the mutations by a plasmid encoding the *neuB-neuC* operon in trans. There is no apparent effect of mutation of *neuB* or *neuC* on the LPS of VC167. We have mutated the corresponding genes in *C. jejuni* MSC57360, the type strain of the O:1 serotype, and there is no effect of mutation of either gene on flagellin or LPS of this strain. However, mutation of a second homolog of a *neuC* gene in MSC57360, termed *siaA*, results in altered mobility of the LPS core and loss of reactivity of the LPS core with cholera toxin. Moreover, there is enhanced immunoreactivity of the core of the mutant with antiserum against whole cells of MSC57360, suggesting that sialic acid functions to mask the LPS core from the immune system. The *neuC* gene of VC167, but not the *neuC* or *siaA* genes of MSC57360, complements a deletion of *neuC* in *E. coli* K1.

**NOTES**

CV8

Serological Analysis of Lipopolysaccharides from *Campylobacter jejuni* Strains That Are Candidates For Use In Prototype Vaccines

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Molecular mimicry between gangliosides and *C. jejuni* lipopolysaccharides (LPSs) has been hypothesized to account for induction of antiganglioside antibodies, which are considered to play a role in GBS pathogenesis. Thus, a successful *C. jejuni* vaccine strain should not contain LPS epitopes that could lead to induction of antiganglioside antibodies. The aim of this study was to serologically characterize the LPSs from two *C. jejuni* candidate vaccine strains, 81-176 and CGL-7, to determine whether ganglioside mimicry was present. Electrophoretic analysis (SDS-PAGE) of phenol-water extracted LPS demonstrated the presence of low-molecular-weight, rough-form LPSs. Colorimetric analysis detected *N*-acetylneuraminic (sialic) acid in LPS from *C. jejuni* 81-176 only. Tests for reaction of LPSs with monoclonal and polyclonal anti-$GM_1$ antibodies were negative in thin-layer chromatography immuno-overlay studies, indicating the absence of $GM_1$ mimicry in vaccine strain LPSs. Antibodies to gangliosides asialo-$GM_1$, $GM_2$, $GD_2$ and $GD_3$, and to disialosyl disaccharides did not react with vaccine strain LPSs but, reacted with a panel of control *C. jejuni* LPSs which are known to bear these ganglioside structures. Results of reactions with cholera toxin (a $GM_1$ ligand) and peanut agglutinin (a Gal$\beta$1$\rightarrow$3GalNAc ligand) indicated the absence of mimicry of major gangliosides by the LPSs. Collectively, the results indicated the absence of mimicry in the vaccine strain LPSs by the major gangliosides implicated in GBS development.

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## Cytokine release by peripheral blood mononuclear cells (PBMC´s) from patients with *Campylobacter concisus* related diarrhoea

CV9

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**Purpose of the study:** To evaluate the cytokine release of PBMC´s from 11 patients (Age & sex) suffering from acute or persistent *C. concisus* associated diarrhoeal disease after stimulation with *C. concisus* sonicate.
**Background:** *C. concisus* is frequently isolated from patients with diarrhoea in our department. In many cases no other pathogenic microorganisms are found in these patients.
**Materials & methods:** PBMC´s of 11 consequtive *C. concisus* patientswere isolated from heparinized blood using "lymphoprep" (Nycomed, Oslo, Norway) and stored in liquid nitrogen until use.
**Results:** PBMC´s from all patients respond with IFN-g after stimulation with *C. concisus* sonicate (mean 432 pg/ml, range 45-906). There are no difference whether the patients have persistent or acute diarrhoea. IL-5 release tended to be higher in PBMC´s from patients with persistent diarrhoea (mean 35 pg/ml, range 22-45) than in patients with acute diarrhoea (mean 18 pg/ml, range 2-39)(p= 0,06). PBMC´s from some patients also produce either IL-4 or IL-10 after stimulation with *C. concisus* sonicate. No difference is seen with regard to duration of the diarrhoeal disease.
**Discussion:** We have shown, that patients with persistent *C. concisus* associated diarrhoea, raises a specific immune response to this bacterium. This supports the hypothesis, that *Campylobacter concisus* can be the causative agent of persistent diarrhoea.

CV10

## Microbiological Outcomes in Volunteers Receiving Experimental Oral Challenge with Live *Campylobacter jejuni*

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NOTES

Live *C. jejuni* was delivered in 1.25% bicarbonate to naïve volunteers as a low (n=5; $10^5$ CFU), mid (n=5; $10^7$), or high (n=5; $10^9$) dose. Five more groups with naïve (n=31) and short-term (~one month) (n=8) or long-term (~one year) (n=7) rechallenged volunteers received only the high dose. The challenge strain (*C.jejuni* 81176) and SOP for preparation (See also Rollins, et al.) and delivery of the inocula were derived from a prior human clinical trial conducted at the CVD (Black, et al.,J.I.D.,1988). Stool specimens were graded 1-5 (1=solid & formed through 5=rice water stool) and assessed for volume and presence of mucus and blood. Organisms were recovered, enumerated and bio- and sero-characterized. In 12 hour increments, mean # of diarrheic stools/Pt. (2.1 +/- 1.4), mean volume of diarrheic stool/Pt. (247.7 cc +/- 141.7), mean # of diarrheic stools with gross blood/Pt. (0.3 +/- 0.2), with occult blood (heme)/Pt. (0.6 +/- 0.5) and with mucus/Pt. (1.4 +/- 0.9) all peaked at 54-66 hours in the high dose naïve patients. Mean volume/diarrheic stool was maximal (185.4 cc +/- 52.4) within the first 24 hours of ingestion and generally declined therafter. In naïve volunteers, maximum colonization was consistently high at all dose levels (range 1.6e6 to >3.0e8 Max. CFU/g; GMT Max. CFU/g = >1.3e8) and persisted until azithromycin was administered. Signs of partial colonization resistance were observed in the short-term (range 0 to 8.3e5 Max. CFU/g; GMT Max. CFU/g = 6.9e3) and long-term rechallenged groups (range 3.3e1 to 7.3e8 Max. CFU/g; GMT Max. CFU/g = 1.4e7). (See also Tribble, et al.)

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CV11

## Genetic diversity of the *Campylobacter* genes encoding immunopositive proteins

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Three *Campylobacter jejuni* 72Dz/92 genes [*cjaA* (*ompHI*), *cjaC*(*hisJ*) and *cjaD*(*omp18*)] coding highly immunopositive proteins are considered to be potential chicken vaccine candidates. The presence and conservation of the *cjaA*, *cjaC* and *cjaD* genes among different *Campylobacter* clinical isolates was determined. The examined genes were detected in thirty *Campylobacter* strains using hybridization as well as Western blot analysis performed with specific anti-CjaA and anti-CjaC antibodies. However, PCR products of the predicted sizes were amplified only from ten out of thirty *Campylobacter* strains tested. Two pairs of primers were designed for each gene. For every *Campylobacter* strain examined the results of the PCR was the same regardless of the employed pair of primers. All strains which were positive with one pair of primers were also positive when the other five pairs of primers were used for PCR assay. The nucleotide sequences of the analyzed genes were compared with the nucleotide sequences of their homologs cloned from other *C. jejuni* strains (M275, A74/0, ATCC29428) as well as with the whole genome sequence of *C. jejuni* NCTC11168. The examined sequences revealed 0 to 16% of divergences. Strain-dependent level of divergence was observed. Among the 4 complete *cjaD* sequences 2 variants were identified which showed 83% identity. Both *cjaC* and *cjaD* sequences from *C. jejuni* M275 are identical to those cloned in our lab.

The polymorphisms detected in *cjaC* were mostly within the 5' region of the gene, while the nucleotide substitutions in *cjaA* and *cjaD* are distributed rather uniformly along the whole gene sequences.

Most of the observed nucleotide substitutions occurred at the third base of the codon. The observed changes are silent mutations which result in no changes in a particular amino-acid or synonymous mutations that result in substitution of amino-acid that does not influence the function of the protein. This observation is consistent with the results of Western blot experiments performed in our lab.

## Immunogenicity of an avirulent *Salmonella enterica* sv. Typhimurium expressing *Campylobacter* genes

CV12

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The immune responses in chickens orally vaccinated with a recombinant avirulent *Salmonella* strains carrying *C. jejuni* 72Dz/92 genes were studied. Three *C. jejuni* genes *cjaA* (*ompH1*), *cjaC* (*hisJ*) and *cjaD* (*omp18*) encoding highly immunogenic proteins were introduced into *E. coli* χ6097 and into avirulent *S. enterica* sv. Typhimurium Δ*crp*Δ*cya*Δ*asd* (χ3987 and χ4550) of two different serotypes (UK-1 and SR-11). pYA3341 Asd$^+$ high-copy number plasmid was used as a cloning vector. The constitutive expression of all analyzed genes was found to be similar in two *Salmonella* strains of different origin. The chickens that were vaccinated at 1 and 14 days of age with $10^8$ cfu of *Salmonella* χ3987 carrying *C. jejuni* genes were used to determine the immune response to heterologous antigens. *Salmonella* χ4072 OMPs and *C. jejuni* 72Dz/92 OMPs were employed as antigens for ELISA tests. CjaA and CjaC proteins are immunologically related. *CjaA* gene product expressed in *E.coli* cells is an outer-membrane located lipoprotein as was showed by Western blotting and labeling with [$^3$H] palmitate. The exact location of CjaC is controversial. 18kDa-*cjaD* gene product, which belongs to the family of peptidoglycan-associated proteins (PALs), is also outer-membrane located protein.

Titers of serum IgG against both used OMPs were higher in fourth than in second week after booster. Titers of IgG antibody in the serum specific for *Campylobacter* OMPs were lower than those toward *Salmonella* OMPs (50-150 vs 100-200, respectively). The distinction was not so suggestive in the case of specific IgA titers in the intestinal washings. There were no significant differences in humoral (serum IgG) immune responses in subgroups of chickens immunized with *Salmonella* strains carrying different *C. jejuni* genes, whereas the subgroup of birds immunized with *Salmonella*/*cjaD* demonstrated lower anti-*Campylobacter* OMPs IgA titers than subgroups of birds vaccinated with *Salmonella*/*cjaA* and *Salmonella*/*cjaC*.

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## Genotype and phenotype diversity amongst *Campylobacter jejuni* isolates from three phage-groups commonly associated with human enteritis in the UK.

CX1

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A longitudinal study of human Campylobacter infections was conducted between January 1990 through to December 1996. A total of 2,491 isolates of *Campylobacter* spp were isolated from human faecal samples at the Preston PHL. All of the isolates were typed using the Preston phage-typing scheme and the five most prevalent phage-groups were (PG52, PG55, PG69, PG90 and PG121). The annual incidence of three well characterised phage-groups was calculated and varied during this seven year study period from 10.9 % to 19.1% for PG52, 3.0 % to 7.0 % for PG55, and 5.7 % to 11.0 % for PG121, with averages of 14.0 %, 4.5 % and 8.3 % respectively. Genotyping of selected *Campylobacter jejuni* isolates from these three phage-groups by RFLP analysis of three gene targets (ribotype, *fla*A type and a random-cloned gene RFLP profile) identified a small number of genotypes amongst these isolates which account for a large proportion of the cases of human infection in the UK. There were significant associations between genotypes, *fla*A profiles, Penner serogroups and Preston phage-groups in strains which were responsible for a significant proportion of human infections during the study period indicating a genetic basis for some of the subdivisions identified by these phenotypic characteristics. This polyphasic approach to understanding the population diversity of *Campylobacter jejuni* has identified three Preston phage-groups which provide a specific marker for strains which are important in sporadic human infections.

CX2 **Molecular Characterization (RAPD and PCR-RFLP) of the *fla* locus of *Campylobacter jejuni* Strains Isolated from a Diarrhea Outbreak in Captive Marmosets.**

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The black-tufted-ear marmoset (*Callithrix pencillata*) is a small non-human neotropical primate extensively used as a biomedical experimental model. This species can be bred (or maintained) in captivity, and diarrhea is an important disease in the animal colonies. In 1987, an outbreak of inflammatory diarrhea by *C. jejuni* ocurred in 47 *penicillata* of different ages and breeding systems. Twenty-nine *C. jejuni* strains were isolated from the symptomatic marmosets. Antimicrobial susceptibility assays, MIC for heavy ions metals, production of enterotoxin and cytotoxins, and the HEp-2 cells chamber-slide adherence/and invasion assays were determined for all the strains. Most *C. jejuni* isolates were non-typable by the Lauwer's serotyping method. We analyzed 19 strains by RAPD techniques (primers 1290, 1283, Wil2 and a pairwise combination of two of these oligonucleotide primers) and PCR-RFLP of the *fla* locus. The fingerprints obtained were numerically analyzed. All generated RAPD and PCR-RFLP of the *fla* locus fingerprints clustered *C. jejuni* strains in two branches; one comprising 15 strains, representing closely related sub-clusters of two pathogenic variants of *C. jejuni*. The 4 remaining strains were not clustered together and could be not associated with the diarrhea outbreak; the source of these 4 strains is unknown. Similar results were found between PCR-RFLP of the *fla* genes and the RAPD using single primers or a combination of two of them. Traditional methods used in this work were not sensitive enough to discriminate the strains related or not with the outbreak. We recommend the use of least two of these molecular techniques to elucidate and to study *C. jejuni* strains associated with outbreaks.

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CX3 **Genetic Polymorphism of the *iam* Locus of *Campylobacter jejuni* by PCR-RFLP.**

**A.C.T. CARVALHO**, M.P. RAMOS-CERVANTES, L.E. CERVANTES and G.M. RUIZ-PALACIOS. National Institute of Nutrition, Mexico City, Mexico.

Using RAPD techniques, we recently characterized a new genetic marker of invasive ness of *C.jejuni/coli*. This RAPD DNA marker of 1.6Kb was preferentially associated with HEp-2 cell invasive strains and was designated *iam* (from "Invasion Associated Marker"). Two putative ORFs (IamA and IamB) were identified in this fragment; IamA shared identities with (several) ATP binding proteins, while IamB shared identities with some hydrophobic hypothetical proteins. PCR amplification of the *iamA* gene was detected in 84% of the invasive and in 22% of the non-invasive strains. At present, we have not been able to detect whether non-invasive strains have an allele for *iam*. Furthermore, RFLP Southern blot hybridization of the *iamA* gene revealed that invasive and non-invasive strains presented an homologous band, but completely polymorphic. To determine wether there are differences in DNA polymorphism between invasive and non-invasive strains, degenerated primers for the *iam* sequence were designed according to: 1) the conserved regions identified in the ATP binding proteins (primers ATP1 and ATP2); and 2) an alignment carried out between the *iam* locus sequence and the DNA of *Helicobacter pylori*, *Escherichia coli* and an 82% homologous DNA sequence found at NCTC 11168 *C. jejuni* genome project. The DNAs of 119 *C.jejuni/coli* invasive and non-invasive strains were amplified at low stringency conditions using forward and reverse primers for the degenerated primers. PCR products were digested with *Hind*I or *Hind*III, respectively. We identified four main PCR-RFLP patterns (H1, H2A, H2B, and H2C) when the p77-p1415 were amplified and restricted with *Hind*III. Most of the PCR- positive (62/66) and invasive (52/66) Campylobacter strains were typed as H1, but the majority of non-invasive and PCR-negative strains was typed as H2b. Using degenerated primers, a PCR-RFLP of the *iamA* and *iamB* genes allowed us to identify invasive Campylobacter strains.

NOTES

CX4

**Genetic differentiation of *C. jejuni* strains isolated from chickens and from patients with gastroenteritis, Guillain-Barré or Miller Fisher syndrome.**

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*Campylobacter jejuni* is the most common cause of foodborne bacterial gastroenteritis. A serious complication associated with *C. jejuni* infection is the development of the neurological disorders Guillain-Barré (GBS) and Miller Fisher syndrome (MFS). An important infection source is the consumption of contaminated poultry products. Strain characteristics of pathogenic *C. jejuni* still have to be established, therefore we studied the clonality among strains infecting chickens and humans. Recently, we described the use of Amplified fragment length polymorphism (AFLP) analysis for high-resolution genotyping of Campylobacter. This method was used for the analysis of 52 strains isolated from patients with uncomplicated gastroenteritis, 50 strains randomly isolated from chickens, 14 strains were GBS-related and 4 strains from patients with MFS. Strains were isolated in the Netherlands and Belgium. All *C. jejuni* strains showed highly heterogeneous fingerprints, that were separated in two genetic lineages (40 % homology). Within these lineages smaller clusters containing human as well as chicken strains are present. Genetic homology (> 90 %) was only observed of 9 gastroenteritis strains with 4 chicken strains and 5 GBS strains. We conclude that AFLP fingerprinting does not detect clonality among *C. jejuni* strains infecting chickens or those causing human infections as gastroenteritis, GBS or MFS. However, within clusters specific bands were identified, that could be of interest for identication of specific strain characteristics.

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CX5

Evidence for a genetically stable clone of *Campylobacter jejuni*

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The genetic stability of selected epidemiologically-linked strains of *C.jejuni* during outbreak situations was investigated using AFLP. Strains isolated in 1998 were investigated from chickens and the environment around three broiler houses which were geographically related. There was little similarity in strains between outbreaks. However, the strains from within all three chicken outbreaks, including strains isolated from the house floor, feed lines, and chicken faeces, were identical at over the 95% level of similarity confirming the genetic stability of these strains within the short time courses of chicken flock outbreaks. Strains were also investigated from a human outbreak, in 1981, thought to be due to contaminated water (Palmer *et al.*, 1983). Three AFLP profiles were recognised from this outbreak confirming the seroptyping undertaken at that time. The major type (serotype P6;L6) isolated from this outbreak was exemplified by strain 81116. This isolate is a well-characterised laboratory strain. Nevertheless the AFLP profile of strain 81116 was identical with all the other P6;L6 strains from the outbreak indicating that it has remained remarkably stable over almost 20 years despite being subtyped on many occasions. Interestingly the AFLP profiles of the P6;L6 strains from the human outbreak and the strains from one of the chicken outbreaks were identical at the 94% level. This similarity is also remarkable and suggests that some clones of *C.jejuni* remain genetically stable in completely different environments over extremely long periods of time and considerable geographical distances.

**CX6** Sequence divergence of the major outer membrane protein of *Campylobacter jejuni*. Q. ZHANG, J.C. MEITZLER, and S. HUANG. Food Animal Health Research Program,The Ohio State University, Wooster, OH.

**NOTES**

The major outer membrane protein (MOMP), a multi-function surface protein of *C. jejuni,* may play an important role in the adaptation of the organism to various environments. Molecular genetic characterization of the MOMP is essential for defining the functions of the protein. In this study, the gene (*momp*) encoding the MOMP protein was initially identified and sequenced from strain 33291. Sequence characterization indicated that the single copy *momp* gene in strain 33291 contains an ORF of 1278 bp. After cleavage of the signal peptide (22 amino acids), the mature MOMP contains 403 amino acids with a calculated molecular mass of 43.5 kDa. MOMP does not share significant homology with any known proteins in the databases including porin sequences from other gram negative bacteria. To analyze the functions and immmunogenicity of the protein, recombinant MOMP was successfully produced in *E. coli*. Immunoblotting analysis revealed that naturally infected chickens produced antibodies to MOMP. Notably, the *momp* alleles in different strains recovered from various animal hosts showed considerable sequence divergence including both substitutions and insertions/deletions. The sequence variations are localized in several regions in the MOMP protein. These findings suggests that the *momp* gene may be used as a genetic marker for molecular typing of different strains of *C. jejuni*. The functional consequence of the sequence divergence in MOMP is being examined in our laboratory.

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**CX7** **Clonality of *Campylobacter jejuni* O:19 Strains Associated with Guillain-Barre Syndrome and Gastroenteritis**. I. NACHAMKIN, J. ENGBERG, A.J. LASTOVICA, M. GUTACKER, P. ARZATE, B.M. ALLOS, M. NICHOLSON, T.W. HO, G.M. McKHANN, J.W. GRIFFIN, and J.C. PIFFARETTI. U. of Pennsylvania, Philadelphia; Serum Statens Institute, Copenhagen, Denmark; Red Cross Children's Hospital, Cape Town; Instituto Nacional Pediatria, Mexico City; Vanderbilt University, Nashville; CDC, Atlanta, GA., Johns Hopkins Univ. , Baltimore; Instituto Cantonale Batteriologico, Lugano, Switzerland.

*C. jejuni* serotype O:19 has been reported to be disproportionately associated with the development of Guillain-Barre Syndrome (GBS). We asked, "is a particular clone (s) of O:19 associated with GBS and do these strains differ from strains isolated from patients with campylobacter gastroenteritis?". Using multilocus enzyme electrophoresis (MLEE), we studied 58 strains of *Campylobacter jejuni*, O:19 (N=39) and non-O:19 (n=19) strains. There were 34 electropherotypes (ETs) among the 58 isolates. For the 39 O:19 strains, there were 15 ETs and for 19 non-O:19 strains, there were 19 ETs. The strains were grouped into 5 main clusters. Clusters A and B contained 36/39 (92.3%) of the O:19 population. Within cluster A, 6 of 9 (67%) GBS associated isolates were contained within three ETs, ET1 (4 GBS strains, 1 enteritis), ET7 (1 GBS strain, 5 enteritis), ET10 (1 GBS strain). Three other GBS associated O:19 strains were contained in the B cluster (2 ETs) that also contained 17 enteritis strains. Clusters C and D contained all 19 non-O:19 strains and preliminarily, 3 O:19 isolates (non-GBS). A fifth cluster, cluster E, contained two O:19 strains, however, with a genetic distance of >0.9, this suggested that these two isolates were not *C. jejuni*. The results of this study show that O:19 strains comprise a unique population that can be differentiated from non-O:19 strains. Further, GBS associated O:19 strains fall into a restricted number of ETs, suggesting that these strains may constitute a subtype within O:19.

**NOTES**

CX8

Flagellin short variable region (SVR) sequence for *Campylobacter* typing.

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Sequence based typing of a fragment of the flaA gene has been proposed for discriminating *Campylobacter* (Meinersmann et al. J. Clin. Micro. 1997;35:2810). This study was undertaken to examine the population genetics and to confirm the epidemiological validity of the test. Ninety-eight strains from 46 independent collections were blind coded along with 11 duplicates of these strains. The code was broken after multiple sequence analysis of 339 bp including the SVR was performed for these strains and the associations were interpreted. Nine of the duplicates were identical and two duplicates differed by 1 bp. Thirty-eight sequence-types were identified that were all epidemiologically relevant. There were two major groups. The members of the largest group (78 isolates) differed from each other by less than 8 % (<28 bp). The second group (20 isolates) differed from the first group by as much as 20 % and differed from each other by 14 % or less (48bp). No isolates from chickens from 24 independent sources were found in the second group. Twenty strains were collected from a single poultry farm. All of the strains collected within the poultry house on that farm had 2 or fewer base differences. Five strains from outside the houses at that farm differed by at least 3% from the house strains and were included in both major groups described above. The differences noted on the study farm could all be accounted for without invoking a hypothesis of recombination. If recombination does play a part in the generation of diversity of the SVR, it appears there must be an unidentified recombination barrier that separates sub-populations of *Campylobacter*.

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CX9

**Variation in the *fla*A gene sequence of *C. jejuni* serotype HS1 clinical isolates.**

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Penner heat-stable serotype 1 (HS1) represents one of the most commonly isolated *C. jejuni* serotypes in the advanced world. It has recently been suggested that HS1 strains may represent a clonal population structure, since PFGE profiles within the group are highly similar, whilst ribotypes and flagellin types also appear highly conserved. This study aimed to investigate the diversity of the flagellin A (*fla*A) gene sequence within Scottish HS1 populations, and to determine whether HS1 sequences formed a discrete group when compared to non-HS1 sequences. A group of 70 HS1 strains, including the HS1 reference strain, were subjected to *fla*A gene typing by *Dde*I-digestion of the full gene. In total, 16 profiles were identified, all similar, although 6 profiles were distinct from the rest, by virtue of possessing a band greater than ~500 bp. The four most prevalent profiles were represented by 24, 12, 10 and 7 strains respectively, with just 1 strain representing most of the remaining 12 profiles. Strains representing each of the 16 *fla*A types were sequenced through their *fla*A genes. These sequences were aligned with an existing database of ~40 *fla*A sequences representing non-HS1 strains. The resultant dendrogram consisted of two main clades, only one of which contained HS1 sequences. The HS1 sequences were related at similarity levels ranging between ~88 to 99.8%, with similar levels of relatedness seen also for the non-HS1 sequences present within the clade. HS1 sequences representing the 6 more distinct *fla*A types (including one of the predominant types) occupied distinct positions within the clade. The apparent clustering of HS1 sequences within just one of the two clades may support a common evolutionary origin. However, with the level of HS1 *fla*A sequence diversity approaching levels described previously for completely unrelated strains, it is clear that care needs to be taken with interpretation of results based on *fla*A sequencing alone.

NOTES

HX1

Genotypes of *H. pylori* isolates from a Himalayan population in India.

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Vanderbilt University[1] and VA Medical Center[2], Nashville, TN; New York University, New York, NY[3], Mayo Clinic, Rochester, MN[4], Delft Diagnostic Lab, Delft, The Netherlands[5], Children's Hospital, Philadelphia, PA[6], Sonam Norboo Memorial Hospital, Leh, Ladakh, India[7].

Although *H. pylori* genotyping has been well-established, there have been few surveys of remote human populations, nor extensive evaluation of *H. pylori* genotypic diversity within a single subject. We studied *H. pylori* isolates from patients in Leh (elevation 3500 meters) in Ladakh, India. Dyspeptic patients were endoscoped, multiple biopsies were obtained and frozen for later culture in the USA. Of 16 adult patients studied, *H. pylori* was isolated from 11 (69%). By serologic assay, of 15 patients tested, 14 (93%) were *H. pylori*$^+$ (11 in the *H. pylori* assay, and 13 in the CagA assay). By PCR, a representative isolate from each of the 11 culture-positive patients was *cag*$^+$. From each of the 11 patients, 2 or 3 biopsies were studied, and in total 182 single colonies were picked, as well as sweeps from each of 24 biopsies. The *vacA* S-type was s1a and s1c for 10 and 1 patient, respectively; each isolate from each patient had the same s-type. For *vacA* m-typing, m1 predominated in 8 patients, and m2a in 3 patients. For *iceA* typing, with only a single exception, all colonies from 7 patients were *iceA*2 and from 4 were *iceA*1. By RAPD-PCR, the isolates from each biopsy from each patient had the same unique pattern. However, in 2 patients, although the predominant pattern was identical, an extra band was consistently seen for colonies from a single biopsy. We conclude that in this population *H. pylori* carriage with *cag*$^+$ strains is universal, and there was no evidence for carriage of more than one strain. However, biopsy-specific clonal variants arose supporting the concept of *in vivo* development of quasispecies.

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HX2

*H. pylori rnr* homologue (HP1248) demonstrates star phylogeny.

R.J. Meinersmann, J. Romero, M.J. Blaser. USDA, Athens, GA; Div. Infect. Dis., Vanderbilt Univ., Nashville, TN

RAPD was used to examine *H. pylori* populations within individuals in Ladakh, India. A patient (#30) was identified in whom isolates from antrum and cardia biopsies yielded an extra band not found in isolates from the fundus biopsy. Sequence analysis showed this band was part of HP1248 (*rnr*-homologue). The lack of a band in colonies from the fundus was due to a polymorphism in the 5' random primer site. Additional polymorphisms were found in *rnr* amongst these strains, but the flanking HP1247 and HP1249 as well as the *recA* and *glmM* genes showed no polymorphisms. Analysis of the complete sequence of HP1248 from 5 strains from various parts of the world showed an average base difference of 5.58 percent, with 226 polymorphic sites, 73 of which were informative. Linkage could be shown for very few of the informative sites, but statistical tests could not document any potential recombination sites. The mean $K_A/K_S$ was 0.37 but tests for selection were unable to support the neutral hypothesis. We then examined 57 *H. pylori* strains (45 human from 5 countries on 4 continents and 12 monkeys) and analyzed sequence polymorphisms for a 231 bp 5' region and a 126 bp 3' region. Neighbor-joining analysis of distances yielded a star phylogeny for both fragments and the monkey and human strains showed no apparent differences. For the 5' fragment, $K_A/K_S = 0.12$, and for the 3' fragment = 0.40, indicating the possibility of differential selection for these two regions of a single gene. In the 5' region, there is an 18 bp island of linkage disequilibrium, and nearly all other informative poylmorphic sites were not linked. These data are consistent with the observations of Suerbaum et al. (PNAS 1998;95:12619) that selective sweeps are rare in *H. pylori* populations, and that the 5' and 3' regions of *rnr* are subject to different selective pressure. Linkage disequilibrium analysis suggests free recombination but the deduced small size of the recombination unit precludes definitive ascertainment of recombination frequency.

**NOTES**

HD1

Use of 3D Modelling of the Urease Holo-enzyme of *Helicobacter pylori* to Characterise the Tertiary Structure and Identify Internal and Surface Exposed Regions.

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**Aim.** We used the amino acid (AA) sequence derived from *ureAB* of *Helicobacter pylori* 26695 (Acc.no.HPAE 000529) to construct a 3D model based on the known structure of the urease of *Klebsiella aerogenes* (pdb entry code 1KAU). The same approach was followed for two *H. pylori* strains, which had different AA sequences for UreB.

**Results.** Protein homology models have been constructed using the Modeler module of the program insightII 98.0. The best models for each sequence were assembled into trimers, using the *Klebsiella aerogenes* 3D structure as a guide, then used for calculations of the per residue solvent accessibility using the NACCESS program (S.Hubbard and J.Thornton 1993).

**Discussion.** Regions of the protein sequence of high solvent accessibility were selected for peptide synthesis and testing for antigenicity. One such region proved to be an exposed loop in a helix-loop-helix motif in the 3D model. By contrast, the Nickel binding site region was found to be of low accessibility but was also selected for antigenicity testing.

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HD2

PREVALENCE OF HELICOBACTER PYLORI INFECTION IN A POPULATION AFTER THE 80$^{TH}$ YEAR OF AGE: A CASE-CONTROL STUDY.

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Epidemiological studies have suggested that the Helicobater pylori (Hp) infection is strictly related to the social and economic characteristics and increases with the age. The peak of prevalance is in the 60$^{th}$ year. Limited information is available on the prevalance of the H.pylori infection in patients after the 80$^{th}$ year of age. Therefore we conducted a case-control study, considering as variable the age. We have enroled 50 patients with high risk of H. Pylori infection. The w. mean age was 91.6 years. The control group was a risk matched population (w. mean was 56.8 years). Patients were tested for serum antibodies to H.pylori; endoscopic investigation with multiple gastric biopsies (2 on antral mucosa, 2 on body mucosa and 2 on duodenal mucosa) and coltural and histological examinations were also performed. A double-blind study was applied to each patient. The gold standard was: Hp positive patients= positive culture or positive histologic examination; Hp negative patients= negative culture and negative histologic examination. The case patients Hp positive were 53%; the control patients Hp positive were 54%; the seropositive case patients were 78%; the seropositive control patients were 78%. No differences were observed for the prevalence between cases and controls: the age is an indipendent variable after the 60$^{th}$ year. The stable value of prevalence after the 60$^{th}$ year of age may be related to several factors as gastric mucosa .ageing-related modifications (bowl metaplasia, atrophy, achlorhydria), repeated cycles of antibiotic therapy, successfully ageing. Our data suggest , according to the available seroprevalence data, that the serodiagnosis is high sensible but low specific.

**HD3** **Rapid Real-time PCR-based Hybridization Analysis of Point Mutations in 23S Ribosomal RNA Genes for Identification of Clarithromycin Resistant *Helicobacter pylori***

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We describe a rapid PCR / hybridization assay for detecting the three mutations in the 23S rRNA gene which lead to clarithromycin (CLA) resistance in *H. pylori*. Genomic DNA is amplified and hybridized to a fluorescently (Cy5) labelled probe in a LightCycler (Roche). Following probe hybridization, the temperature within the reaction capillary is increased and the Cy5 fluorescent signal, produced when the probe is annealed, is monitored by a fluorimeter integral to the LightCycler. Where there are mismatched bases between the probe and target, the melting temperature occurs at a lower temperature than that of a perfectly matched hybrid. Our probe was complementary to the gene sequence of the clarithromycin sensitive phenotype. 100 strains of *H. pylori* were examined. 34 strains from 25 patients were resistant to clarithromycin (MIC of >2mg/l). Probe-target melting temperatures were: 68°C for all 66 strains that were sensitive to CLA in standard antibiotic assays and 1 / 34 resistant strain (no mutation detected, on sequencing, in this strain); 58°C for 31 /34 resistant strains (A2143G and A2144G mutations detected by PCR-RFLP analysis for these 31 strains); 63°C for 2 / 34 resistant strains (A2143C mutation detected on sequencing). This PCR-based hybridisation assay, which is performed in a single, closed reaction capillary and which takes less than one hour, could be applied directly to biopsy material.

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**HD4** *Helicobacter pylori* infections in patients with gastroduodenal ulcers and gastric malignancies in Karachi , Pakistan.

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*Helicobacter pylori* has emerged as a major risk factor in the causation of peptic ulcer disease, gastritis and gastric cancer in humans. Genotypic/ phenotypic variations in isolates from different countries as well as host response to infection with specific immune mechanisms may affect the severity of disease and its progression to gastric cancer. This study was initiated to determine the prevalence of *H pylori* infection in patients with gastroduodenal ulcers and gastric malignancies. Blood samples and gastric biopsies collected from patients (n=327) and matched controls (n=100) were processed for *H pylori* infection by Culture, Histology , Helicourease (IIDRL , Pakistan), anti–H pylori IgG by ELISA and H flex . *H pylori* infection was found to be more prevalent in duodenal ulcer patients( 82%) than those with duodenitis ( 68%), gastric ulcer ( 65%) and endoscopically normal controls ( 35%). The overall prevalence of *H pylori* infection was 53-67% by the invasive tests including culture( 53%), histology (67%), and Helicourease(65%) however, the non- invasive tests like ELISA and H flex gave a much higher infection rate.Ten out of15 confirmed cases of cancer were positive for *H pylori* specific antibody(67%). The median specific IgG was higher in the cases than the controls ( 98ugs /ml vs 4.5 ugs/ml). Out of the 26 close family members of cancer patients, 20 showed high titer of anti *H pylori* antibody in their sera. Since *H pylori* infection is highly prevalent in Pakistan , an early diagnosis, proper treatment, healthy living conditions may reduce the prevalence rate as well as intrafamilial transmission of infection and its progression to cancer in some individuals in our community.

HD5

## Zoonotic helicobacter: "*H. heilmannii*" , a human gastric pathogen, and *H. bizzozeronii*, a frequent canine gastric coloniser, represent the same species

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"*H. heilmannii*" (also called "*Gastrospirillum hominis*") accounts for approximately 1% of gastritis cases in humans. *H. bizzozeronii* accounts for almost 50% of *Helicobacter* species cultured from dogs. Canine-human transmission of "*H. heilmannii*" has long been suspected but until recently attempts to culture the organism have been unsuccessful. The recent culture of a human "*H. heilmannii*" strain in Denmark provided the opportunity to determine its taxonomic relationship to ultrastructually similar helicobacters. A detailed comparative analysis of the phenotypic characteristics, whole-cell protein profile, 16S rRNA gene sequence and DNA-DNA homology of the human isolate to other helicobacters was undertaken. The human strain differed phenotypically from *H. bizzozeronii* only in exhibiting tolerance to bile. Phylogenetic analysis clearly demonstrated that the human isolate, *H. bizzozeronii*, other "*H. heilmannii*" strains and closely related species *H. felis* and *H. salomonis* form a highly related group. Numerical analysis of protein profiles clustered the human strain with those of *H. bizzozeronii* type and reference strains. DNA-DNA hybridisation experiments showed the human isolate was highly related to *H. bizzozeronii*, with lower hybridization values obtained with *H. salomonis*, the next most closely related species. We conclude that the cultured human strain examined is a *H. bizzozeronii* isolate and that this species can be transmitted from domestic pets to humans, a finding with clear public health significance.

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## Detection of *Helicobacter suis* in gastric samples of pigs by PCR: comparison with other invasive diagnostic techniques

HD6

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Recently a new 16S rDNA based PCR-assay was developed for the specific detection of *Helicobacter suis* (former *"Gastrospirillum suis"*) in porcine gastric samples. In this study, this PCR – assay was compared to 3 other invasive diagnostic methods (rapid urease test, immunohistochemistry, giemsa stained histology). The rapid urease test was evaluated after two different time intervals, 3 hours and 20 hours post inoculation (p.i.). Antral stomach samples from 200 slaughterhouse pigs from Belgium and The Netherlands were examined. Bacterial presence was determined in 77 % of the samples by PCR in combination with southern blot hybridisation (154/200), 56 % by immunohistochemistry (111/200), 61 % by urease testing (20 h p.i.) (122/200), 36 % by urease testing (3h p.i.) (71/200) and 33 % by giemsa staining (65/200). Sensitivity, specificity and concurrence values were assessed for each test by pairwise comparisons between tests. Microscopic detection of immunohistochemically-labeled or giemsa stained *H. suis* cells in stomach sections proved to be highly specific (100 %) but relatively insensitive (72 %, 42 %) as compared to the PCR-assay. A longer incubation time of the urease test improved sensitivity considerably (74 % vs. 55 %) but was accompanied by a loss of specificity (72 % vs. 93 %). In conclusion, we found the *H. suis* - specific PCR assay to be a sensitive and reliable diagnostic method for the detection of *H. suis* in the stomach of pigs, making it an interesting tool for future *H. suis* based research.

HE1

## PCR DETECTION OF HELICOBACTER PYLORI (H. PYLORI) IN WATER SAMPLES COLLECTED FROM RIVERS IN JAPAN

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H. pylori infects the gastric mucosal membrane and is thought to be causatively involved in tumorigenesis via primarily developing gastritis, chronic gastritis, gastric atrophy, and duodenal ulcer. Although drinking water derived from rivers is suspected as the main source of H. pylori infection in humans, much remains to be experimentally demonstrated for proof. In the present study, we examined, using PCR method, H. pylori contamination in water samples collected from rivers. The results are presented below.

Eight of the 62 samples (12.8%) were positive for producing a DNA band corresponding to 382 bp-PCR products when subjected to PCR using a pari of primers W-43 and W-45. When a pair of primers W-42 and W-45 were used for PCR, all samples were negative for expected PCR products. Finally, 3 of the 8 samples (4.8% of the total cases) were shown to be truly positive for H. pylori as demonstrated by Southern blot analysis.

The results in the present study strongly suggested for the first time that river water in Japan is contaminated by H. pylori.

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HE2

## A Strain-variable Locus in *Helicobacter pylori* Displays Dynamic Equilibrium *in vivo*

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There is growing evidence that rearrangements occur in the genomes of *H. pylori* strains during infection. To determine whether such rearrangements could be identified in epidemiologically related isolates of *H. pylori*, ten sets of paired isolates (i.e. isolates obtained from the separate biopsies taken from the same patient during a single endoscopy session) were screened by PCR for the presence of a recently described, strain-variable, tRNA-associated locus (*trl*) [Dundon *et al.* Microbiology (1999) 145:1289-1298]. The analysis revealed that in one of the sets of paired isolates one of the isolates was *trl*-positive while the other was *trl*-negative. The genetic relatedness of these two isolates was investigated by RAPD and RFLP analysis of the *cag*A and *fla*A loci. Similarity analysis of the profiles generated confirmed that the isolates were clonally related. This study, therefore, provides evidence for a recombinational event occuring at this specific locus within clonally related isolates and suggests that a dynamic equilbrium exists between the loss (or acquistion) of *trl* in certain isolates of *H. pylori*.

HE3

**Risk Factors Associated With *Helicobacter pylori* (Hp) Infection In Young Mexican Children.** V. LUQUEÑO, A. SOLANO, Z. L. BARAJAS, M.L. GUERRERO, G. M. RUIZ-PALACIOS. Instituto Nacional de la Nutrición, Mexico City, Mexico.

**Background**: Hp infection has a worldwide distribution. Even though infection frequently occurs at an early age, epidemiologic studies have focused on risk factors in older individuals. **Objectives**: To determine the prevalence and risk factors associated with Hp infection in children under 3 years of age in a periurban area of Mexico City. **Methods**: A population-based, cross-sectional survey of 264 children, aged 12 to 36 months, was conducted from September through November 1996. Seropositivity for Hp was correlated with socio-demographic and hygiene characteristics among families of the children. Sera were tested for IgG antibodies to Hp with an inmunoassay (ELISA), and corroborated with Western Blot. Risk were estimated using a regression model. **Results**: 65 (25%) children were infected with Hp. Prevalence of Hp infection increased with age ($P$=.02). Risks factors adjusted for age were:

| Variable | | OR | 95% C.I.. | $P$ |
|---|---|---|---|---|
| 1. Gender: Male | | 1.00 | | |
| Female | | 1.75 | 0.93- 3.28 | 0.07 |
| 2. Housing: Nuclear family in a household | | 1.00 | | |
| Nuclear famliy living in one room | | 4.10 | 1.27-13.83 | 0.01 |
| Two or more families per household | | 3.30 | 1.29- 8.42 | 0.01 |
| 3. Drinking water at easy reach of child: | No | 1.00 | | |
| | Yes | 2.18 | 1.15-4.13 | 0.01 |
| 4. Cooking vegetables before consumption: | Yes | 1.00 | | |
| | No | 2.18 | 1.15-4.13 | 0.04 |
| 5. Children in close contact with puppies: | No | 1.00 | | |
| | Yes | 3.28 | 0.90-11.97 | 0.07 |

**Conclusions.** There is a high prevalence of Hp infection among Mexican infants. Household crowding, drinking contaminated water, eating raw vegetables, being female, and having close contact with puppies might play an important role in the transmision of Hp.

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HE4

Phylogenic relationship of *Helicobacter heilmannii*-like organisms originating from humans and animals.

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Of the unofficial *Helicobacter* species *"G. hominis"* or *"H. heilmannii"*, appears to be the dominant organism found in a range of animal species including primates, pigs, cats and dogs. Further study of these bacteria has been hampered due to difficulties in growing these bacteria *in vitro;* however, we have maintained a number of these isolates *in vivo* in mice. **Aim**: to examine these bacteria obtained from human patients (n = 4), non-human primates (crab eating macaques, rhesus macaque, mandrill monkey) and a bobcat. **Method**: DNA was extracted from gastric mucus of SPF mice colonised with either biopsies from patients or mucus from animals. *Helicobacter* 16S ribosomal DNA was amplified using two *Helicobacter* specific PCR's. Partial 16S rDNA sequences were analysed with the Clustalw and PHYLIP programs **Results**: The current study corroborates a previous study of two of the human isolates which involved cloning 16S rDNA rather than identification by direct sequencing. These two human isolates were less than 97% similar with one of the isolates (Human 2 – clone G2A9) clustering with culturable gastric helicobacters (*H. felis, H. salomonis & H. bizzozeronii).* Two additional human isolates and those from the non-human primates formed a distinct cluster with Human 1 (clone G1A1). The sequences of these 8 isolates were highly conserved with less than 1% difference and this cluster was distinct from that which included Human 2. **Conclusion:** the above results may possibly point to a dominant species of gastric spirilla. Further studies looking at other genes such as that encoding urease is currently underway. A combination of these molecular and morphological analyses may provide evidence for a coherent species *novo* within *Helicobacter.*

**HE5** Examination of Geographic, Clinical and Intrahost Variation in the 3' Repeat Region of *cagA* SK KIM, J ROMERO, T. ANDO, E NG, R PEEK, MJ BLASER. Kyungpook University Medical Center, Taegu, Korea; Vanderbilt University and VA Medical Center, Nashville, TN

**NOTES**

The cryptic gene *cagA* is a marker for *H. pylori* strains with enhanced interaction with epithelial cells. The 3' portion of *cagA* has several repetitive elements that account for the variability in the size of the encoded CagA protein from 120-140 kDa. Previous studies have shown allelic variation affecting 5' regions of *cagA*. Other studies, examining variability in the 3' repeat region (3' RR) have suggested that these may relate to clinical differences in outcomes of *H. pylori* carriage. To examine whether 3' RR variation is related to geographic or clinical differences or differing locations in the stomach, we studied 116 *cagA*$^+$ strains from 4 countries [Japan (n=28), Hong Kong [China] (n=25), India (n=27), USA (n=36)], using PCR primers cag3/cag4 (from Covacci et al) and cag5/cag6 (Yoshioka et al). The cag5/6 primers had a broader range than cag 3/4 amongst these geographically diverse isolates. Of individual isolates from 78 patients, 68 (87.2%) showed a 650bp band, 8 showed bands 50 or 100bp greater, and 2 showed bands 50bp shorter. For the patients with DU (n=32), NUD (n=32), and gastric cancer (n=14), there was little variation in the rate (12.5-14.3%) at which the size polymorphisms were seen, and there was no geographic trend. For 13 other USA or India patients, 31 isolates from 2 or 3 gastric biopsies/patient were studied; 30 showed the 650 band, but from one USA patient, an antral isolate yielded a 550bp band. RAPD PCR showed identical patterns for the antral and cardia isolate, indicating that they were clonal variants of one another. Study of single colonies from the antrum and cardia showed that each of the 3 antral isolates yielded a 550bp band, whereas the 5 cardia isolates yielded a 650bp band. These studies provide evidence against geographic or clinical association with 3'RR variation, but indicate that diversification is continuing *in vivo*, with no obvious relation to particular gastric sites.

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**HE6** *Helicobacter pylori* and Drinking Water: Occurrence, Persistence, and Clinical Infection

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Epidemiological studies have suggested a possible role of contaminated water in the transmission of *Helicobacter pylori*. We have examined this possibility in field and laboratory studies on the occurrence and persistence of *H. pylori* in groundwater. Two independent detection techniques -- fluorescent antibody-tetrazolium reduction (FACTC) and a novel semi-nested PCR method were used to survey water samples for the presence of *H. pylori*. The FACTC method allows direct microscopic enumeration of metabolically active *H. pylori* while the nested PCR method allows for an independent detection of a species-specific sequence of the *ureA* gene. In surveyed well water samples, there was a statistically significant (Fisher's Exact Test $p < 0.02$) between the presence of *H. pylori* in well water and infection in individuals consuming the water. *E. coli*, was found in the majority, but not all of the wells in which *H. pylori* was detected strengthening the argument for a fecal-oral route of transmission.

Laboratory studies examined the persistence of *H. pylori* in groundwater and the effect of common water disinfectants on *H. pylori*. In low nutrient synthetic groundwater, culturable *H. pylori* showed a rapid decline in the number of culturable cells. The addition of either acetate or synthetic sewage was found to significantly increase the persistence of *H. pylori* in groundwater microcosms indicating that organic matter can have a significant impact on the survival of culturable organisms in these low nutrient environments. In addition, *H. pylori* was found to be more resistant to oxidizing disinfection agents (chlorine, chloramine, ozone) that either *Campylobacter jejuni* or *E. coli*. Therefore, *H. pylori* may be capable of entering into and surviving within water distribution systems.

Natural transformation of *Helicobacter pylori* HE7

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*Helicobacter pylori* exhibit a high degree of genetic diversity among strains and many strains are naturally competent for transformation by exogenous DNA. However, the mechanism by natural transformation occurs in *H. pylori* is not well understood. To investigate this phenomenon, we first tested whether there were barriers to transformation with regard to the *H. pylori* strain from which the donor DNA was derived, how much DNA is required to reach the maximum transformation frequency, whether the addition of *H. pylori* or non-*H. pylori* DNA could inhibit transformation, and whether the genes of the *cag* pathogenicity island are required for transformation. Five *H. pylori* strains with spontaneous resistance to streptomycin were selected for use as sources of DNA. The transformation frequency varied as much as 34-fold between wild type recipient strains ($7 \times 10^{-6}$ to $2.4 \times 10^{-4}$ transformants/μg DNA/cfu). However, each recipient strain showed little difference in transformation frequency, regardless of whether the source of DNA was homologous or heterologous to that strain. Additionally, analysis of dose-response experiments with strain HPK5 and purified DNA from the homologous streptomycin resistant mutant showed the maximum transformation efficiency to be approximately $5.2 \times 10^{-3}$ transformants/μg DNA/cfu. When strain HPK5 was transformed with DNA from the homologous streptomycin resistant strain, the addition of homologous *H. pylori* competitor DNA was found to decrease the transformation frequency in a dose-dependent manner, while the addition of *E. coli* competitor DNA had little effect. Finally, deletion of the *cag* island did not reduce the frequency of transformation. These studies indicate that few, if any, barriers exist to transformation of *H. pylori* by chromosomal DNA from heterologous *H. pylori* strains, that DNA transformation has rate-limiting steps, that *H. pylori* is able to distinguish *H. pylori* DNA from *E. coli* DNA and that the genes of the *cag* island are not required for transformation.

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**Insertion Elements in Intergenic DNA-Regions of *Helicobacter pylori*** HE8

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The DNA sequence of single genes as well as the gene order varies considerably among *Helicobacter pylori* strains. It was the aim of this study to investigate genetic diversity especially in noncoding DNA regions flanking the right end of pathogenicity island (PAI) cagI, and in front of the genes *ribA* and *vacA*. The DNA regions of interest were analysed by PCR amplification. The reference strains NCTC11638, 26695, G27, P1, 151, 2012, as well as 158 clinical isolates were analysed. In front of the *vacA* gene, small insertion (IS) elements which are around 50-100 Bp in size, were detected by variations in the size of PCR products. Among the strains analysed five subtypes could be differentiated. The intergenic region in front of *ribA* allowed also differentiation of strains in five subtypes as shown earlier. Sequence analysis and DNA hybridization indicated the presence of insertion elements which were present in single copy. The elements were not detected in the genome of strain 26695. The IS element found in strain NCTC11638 is completely conserved but located at another site in strain J99. Extensive genetic diversity was also found for the DNA region downstream of the cagI PAI which was described earlier for strains from the US. Four subtypes could be differentiated. Sequence analysis revealed the presence of IS605 or IS606 homologous DNA in most strains. In case of one strain, P1, the inserted DNA did not match any other sequence present in databases. The results demonstrate that insertion elements in the noncoding DNA between genes contribute to the genetic diversity of *H. pylori*. The different location of the NCTC11638 IS element in strain J99 provides evidence that the element is mobile. The new IS elements described might be of use for strain typing, analysis of genetic heterogeneity of *H. pylori*, and for epidemiological purposes.

HE9 *Helicobacter* Phylogenetic Diversity by 16S rRNA Sequence Analysis

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Currently there are 18 validly described *Helicobacter* species. In our studies over the past nine years, 16S rRNA sequences have been determined for approximately 225 helicobacter isolates from 15 mammalian species, including human, cat, dog, ferret, mouse, rat, gerbil, hamster, mastomys, woodchuck, pig, rhesus monkey, cottontop marmoset, and dolphin, and from 3 bird species, tern, gull and house sparrow. These sequences cluster into over 50 phylotypes that differ by greater than 1 percent sequence difference. Organisms within a phylotpye have sequences that differ by less than 1 percent. These phylotypes generally correspond to *Helicobacter* species, but this must be proved in each case by extensive taxonomic studies prior to formal naming. The genus *Helicobacter* includes cultivable and not-yet-cultivated members. The genus also includes organisms with specific ultrastructures that have been called "*Flexispira*" and "*Gastrospirillum*". Organisms with "*Flexispira*" morphology represent at least 8 distinct phylotypes or species of helicobacters. There are at least 4 distinct phylotypes or species of "*Gastrospirillum*". The genus *Helicobacter* is extremely diverse, and, as additional host species are examined, the detected diversity is sure to increase.

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HE10 **Immune response to *Helicobacter pylori* antigens in Bangladeshi children and adults in two orphanages defined by different serological analyses**

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Prevalence rates for *H. pylori* infecting Bangladeshi subjects of up to 92% have been reported previously. Our aim was to investigate the prevalence of infections in young individuals living in close proximity in orphanages, to determine frequency of infections due to CagA producing strains and to evaluate the reliability of a commercial ELISA for such a study population. Serum samples were collected from 93 subjects living in two orphanages that comprised 68 orphan boys aged up to 18 years, and 12 adult carers and their families (25 subjects). All subjects were interviewed and for each a questionnaire was completed. Serum samples were examined by ELISA (Premier Launch kit) and anti-CagA antibody was detected by immunoblotting (IB) using strain 26695 (NCTC 12455) as the antigen (whole cell extract). Immunoblotting gave overall prevalence rates of 79% (weak responses excluded) and 96% (all responses) compared to 84% by ELISA. When weak positives were excluded, the two tests showed 90% correlation giving a prevalence of 79% (IB) for the orphans. The nine discrepant results were mostly for children aged 12 to 14 years. CagA prevalence was 71% for all seropositives and 86% for the strong seropositives and was similar to that reported for UK subjects.

## Lectin typing of *Helicobacter pylori* from Various Geographical Regions HE11

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We have previously developed a phenotypic typing system for *Helicobacter pylori* strains from an Irish population using lectins which bind the bacterium's LPS. The present study was undertaken to assess the lectin types of *H. pylori* strains from other geographical regions. Strains were isolated from predominantly European (95%) and also non European (5%) countries. In this study 306 strains were tested. LPS-enriched samples, for use in the assay, were prepared by proteolytic degradation of whole cell extracts. The samples and lectins (*Erythrina cristigali*, *Lotus tetragonolobus* , *Ulex europeas I* and *Triticum vulgaris*) were mixed in microtiter wells and agglutination was determined visually with lectin types assigned according to our previous scheme. Of the 16 possible lectin types (MH1-16) at least one example of each type was observed The most common lectin type was type MH1 which reacts with every lectin in the panel. MH10 was the dominant lectin type for the largest group of strains in the study which were Swedish (89/306), and MH5 was the dominant lectin type in Estonian strains. However, for every other country in the study (14 countries) MH1 was the dominant lectin type. Each country in the study had an individual frequency pattern for the lectin types of their strains. French and Swedish strains displayed the most heterogeneity of lectin types. Furthermore, type MH16 which does not react with any of the lectins was more frequently observed in Swedish and Portuguese strains, probably due to extensive passage of these strains. The diversity of lectin types indicates variance among strains of *H. pylori* in the expression of certain sugars on their LPSs. Nevertheless, the dominance of type MH1 in many geographical regions indicates that the expression of certain glycosylated structures is conserved amongst strains of *H. pylori*.

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## Variable detection rates of the *cagA* gene in *Helicobacter pylori* strains from dyspeptic Iranian population HE12

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**Introduction:** Cytotoxin-associated gene A (*cagA*) positive *H. pylori* strains have been repeatedly reported to be more prevalent in patients with peptic ulcer disease than those with non-ulcer dyspepsia. Thus, this gene is used as a virulence marker to detect high risk patients. Detection of such patients is highly essential in developing countries where nearly 80% of the population is infected. In this study, prevalence of *cagA*-positive strains is determined using different sets of published primers.

**Material & Method:** Gastric biopsies were obtained from dyspeptic patients and tested for *H. pylori* infection by culture and rapid urease test. *H. pylori* DNA was extracted from the biopsies and screened for *cagA* gene using primer sets reported by Peek et al. 1995 and Vander Ende et al. 1996.

**Results:** Depending on the primer sets used for PCR, the prevalence of *cagA*-positive strains is significantly different.

| Primers | NUD | PUD | NUD & PUD | p value |
|---|---|---|---|---|
| cagA-200 | 86% | 88% | 87% | 0.71 |
| cagA-350 | 75% | 67% | 73% | 0.67 |
| cagA-570 | 24% | 35% | 31% | 0.07 |

**Conclusion:** The *cagA* detection rate has an inverse correlation with the size of the amplified region in the gene. In addition, the association between *cagA*-positive strains and peptic ulcer disease is variable depending on the primer sets used.

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**HE13** **Variable detection rates for *Helicobacter pylori* infection in Iranian population amplifying different conserved genes**

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**Introduction:** The prevalence of *H. pylori* infection is commonly determined by amplification of *H. pylori* conserved genes such as the *ureB*, *ureC*, *hspA*, etc. This methodology had proved to be reliable screening patient populations from western countries. In this study, *H. pylori* infected patients were screened by PCR amplification of four conserved genes. **Material & Method:** Gastric biopsies were obtained from dyspeptic patients and tested for *H. pylori* infection by culture and rapid urease test. *H. pylori* DNA was extracted from the biopsies and screened by PCR using published primer sets for *ureB*, *16SrRNA* (valentine et al.), *ureC*, and *hspA* (*ureC* and *hspA* primers were a kind gift from Dr. A. Labigne, Pasteur Institute of Paris). **Results:** Depending on the amplified gene the detection rates for *H. pylori* were significantly different. **Conclusion:** Detection of *H. pylori* infection in developing countries with high prevalence of infection should be performed by amplifying more than one conserved gene. In this regard, amplification of *ureC* and *hspA* gene gives most optimal result. In addition, the variable amplification of conserved genes from *H. pylori* isolates indicates a vast heterogenity among different strains and disparity from western *H. pylori* strains. Table: (ø ) Both positives, (*) Either positive

| Gene | hspA | ureB | ureC | 16SrRNA |
|---|---|---|---|---|
| hspA | 77% | 81%* | 100%* | 78%* |
| ureB | 46%ø | 77% | 93%* | 81%* |
| ureC | 78%ø | 71%ø | 92% | 92%* |
| 16SrRNA | 13%ø | 9%ø | 41%ø | 35% |

**HE14** **IgG Antibodies against *Helicobacter heilmannii* in blood donors**

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**Background**: *H. heilmannii* (Hh), has been described in histological sections and recently it has been cultured from human gastric mucosa. The bacterium is less frequently found in stomach of patients with gastritis than *H. pylori* (Hp). **The aim** of this study was to measure antibodies against *Hh* by ELISA technique, in blood donors. **Materials and Methods**: Serum samples were obtained from 281 blood donors (mean age 31.7 years, range 18-62 years; 20 female, 261 males) who admitted Hacettepe University Hospital. Separate ELISA plates were coated with *Hh* antigens and *Hp* antigens. Serum samples were absorbed with *Hp* antigen and added to the ELISA plates with *Hh* antigen in duplicate and incubated. The plates were, washed and incubated with HRP-conjugated antibodies to human IgG. The plates were washed and developed with $H_2O_2$ + TMB. The chromogenic reaction was read at 450 nm, using 620 nm as reference. All serum samples were tested for *Hp* antibodies, by the same procedure, except the sera samples were absorbed with *Hh* antigen. **Results**: For *Hh*, the lower cut-off level was estimated to 0,4 and upper cut-off level to 0,5. Using these levels 17 out of 281 (6 %) were positive and 13 (4.6 %) were in the intermediate range. The antibody levels to *Hh* had a screwed normal distribution, without a double bulge as seen for antibody responses to infections with very low prevalence. The *Hp* prevalence at 84 % was similar to previous reports. **Discussion**: The data on *Hh* is expected to be specific for *Hh* as the sera has been absorbed with *Hp* antigen, and all sera from *Hh* patients are above the cut off value. It can not be excluded that the increased values could be due to cross-reactions with *H. felis*, intestinal *Helicobacter* or Campylobacter species because this has not yet been investigated in details.

## Correlation between *H. pylori* genotypes, production of vacuolating cytotoxin and developing disease

HE15

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Hp is associated with chronic active gastritis, ulcer disease, adenocarcinoma or MALT-lymphoma. Bacterial virulence factors like the vacuolating cytotoxin (VacA) and the cytotoxin associated antigen (CagA) may contribute to these gastric diseases.
**Methods**: Fifty-one Hp strains from patients with gastritis (n=16), gastric adenocarcinoma (n=12), MALT-lymphoma (n=11) and ulcus duodeni (n=12) were screened for the presence of the allelic subtypes of the 5' (s1,2) and middle (m1,2) region of the VacA gene and for different regions of the CagA pathogenicity island (CagAc, CagAm, picB) using PCR. In addition supernatants of Hp broth cultures were tested in a VERO-cell culture assay. **Results**: Vacuolisation in VERO-cells was seen in 22/51 Hp isolates (gastritis 6/16, gastric adenocarcinoma 8/12, MALT-lymphoma 2/11, ulcus duodeni 6/12). Strong correlations were seen with VacA genotype m1 and s1m1, CagAc and PicB and results obtained in the VERO-celltest ($p<0,02$ to 0,04). VacAs1m1 ($p<0,08$) and CagAc ($p<0,07$) genotypes were correlated to gastric adenocarcinoma, VacAs2m2 ($p<0,06$) to MALT-lymphoma, and CagAc ($p<0,07$) and PicB ($p<0,07$) to duodenal ulcer disease. **Conclusions**: The production of cytotoxins was correlated to gastric adenocarcinoma, whereas a negative correlation to the development of MALT-lymphoma was determined. Patients with Hp strains positive for the VacAs1m1 genotype and positive in a VERO-cell assay are more likely to develop gastric adenocarcinoma. Cytotoxin-positive Hp strains with the genotypes CagAc and PicB, respectively, are correlated to duodenal ulcer disease.

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## *cagA* Status &*vacA* Subtype are NOT Associated with Disease Status but do Differ Significantly in Patients of Different Ethnic Origins

HE16

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**Background**:*cagA* and*vacA* have been reported to be associated with more serious disease development. In particular, *cagA* and*vacA* s1a and to a lesser extent *vacA* s1b subtypes have been associated with peptic ulceration (PUD). Current evidence suggests that geographic variations in *cagA* &*vacA* subtypes exist however it is unclear how such variations in subtype relate to disease association. **Aim:** To examine the*vacA* heterogeneity and *cagA* status of *H. pylori* strains isolated from subjects of different ethnic origin & disease profile. **Methods**: Antral biopsies collected from 25 symptomatic subjects of Vietnamese, Greek and Lebanese origin were cultured on CSA agar, subcultured once after which 6-14 colonies were selected for further culture. Colonies were then harvested and DNA extracted. Analysis of *cagA* and *vacA* subtype was investigated by PCR. **Results:** The *cagA* status and *vacA* subtype of patients is shown below.

| Ethnic origin | No.sub-types (no. subjects) | *cagA* +ve | *vacA* subtypes | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | s1a | | s1b | | s2 | |
| | | | m1 | m2 | m1 | m2 | m1 | m2 |
| Vietnamese n=10* | 1 (10) | 70% | 2 | 6 | | | | |
| Greek n=9 | 1 (7) | 54.5% | 5 | | 1 | | | 1 |
| | 2 (1) | | 1 | | | | | |
| | 3 (1) | | 1 | | | | | |
| Lebanese n=6 | 1 (5) | | | 1 | 1 | | 1 | 2 |
| | 2 (1) | 14.3% | | | | | 1 | 1 |

.* 2 patients had an s1a subtype but were untypable for the m region

The predominant *vacA* subtype in subjects of Vietnamese origin was s1a/m2, of Greek origin s1a/m1and Lebanese origin s2/m2 and s2/m1. NO association was found to exist between either *cagA* status or *vacA* subtype and peptic ulcer disease. **Conclusions:** Major differences exist in both the *cagA* prevalence and *vacA* subtype in patients of different ethnic origin. This represents the first report of a s2/m1 strain.

HE17 **HELICOBACTER SPECIES DETECTED IN LIVER SAMPLES OF PATIENTS WITH CHOLESTATIC DISEASES.**

NOTES

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Two new *Helicobacter* species (*H hepaticus* and *H bilis*) have been shown to cause chronic liver diseases in laboratory rodents. Other new urease positive and negative *Helicobacter* species have been reported to cause similar diseases in animals such as dog (*H canis*), hamster (*H cholecystus*), sheep (*H rappini*) and humans. We diagnosed *Helicobacter* possibly causing sclerosing cholangitis and biliary cirrhosis in Swedish patients (n=24). By PCR on liver tissue, using primers specific for *Helicobacter* genus, 20 samples were positive. In a group of non-cholestatic liver cirrhosis (NCLC) patients (n=13) 1 sample was positive and in a group of 8 healthy individuals none was positive. By strain specific PCR, 9 samples were identified as *H pylori* and 6 as *H rappini*. The specificity of the *H pylori* PCR-positive samples was confirmed by Southern blot hybridization. In an immunoblot assay, based on *H hepaticus* and *H pylori* surface proteins, some preliminary results from a first study of patients with various forms of cirrhosis will be presented. Our data suggest that some strains of *H pylori*, probably adapted to survive in human bile, can infect the human liver and the bile tree. Culture for such organisms from liver and bile tract biopsies have so far been negative indicating that specific nutrients are required for growth. Gene regulation allowing *Salmonellae* to grow in bile has recently been described. A similar strategy for *Helicobacter* to grow in the human small and large bowel and the liver is proposed.

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HE18 **Genetic Diversity in *Helicobacter pullorum* from Human and Poultry Sources Identified by an Amplified Fragment Length Polymorphism Technique and Pulsed-Field Gel Electrophoresis**

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In 1994 *H. pullorum* was described as a novel species that could be isolated from the faeces and carcasses of asyptomatic poultry. The organism has also been associated with human gastroenteritis. The aim of our study was to develop methods that could be used as epidemiological tools and to examine interstrain genetic diversity within *H. pullorum*. Two fingerprinting techniques were employed: amplified fragment length polymorphism (AFLP) and pulsed field gel electrophoretic analysis. The 20 strains examined were from four countries and comprised 13 human isolates and seven poultry isolates. Their identity was confirmed by a species-specific PCR assay.

Most strains showed a high degree of diversity with the two genotyping approaches. However, both methods indicated a clonal origin for two strains from the same poultry flock and established a close relatedness between three chicken carcass isolates from one processing plant.

We conclude that these two genotyping techniques will provide a useful basis for future epidemiological investigations of *H. pullorum* in poultry, and may provide a link with its possible causal role in human gastrointestinal infections.

## Evidence That *cag*$^+$ *H. pylori* Strains Are Disappearing More Rapidly Than *cag*$^-$ Strains During Modernization

HE19

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The prevalence of *H. pylori* colonization in developed country populations has been declining, as shown by comparison of community-based surveys of adults the town of in Vaamala, Finland in 1973 and in 1994, using stored sera and assessing IgG and IgA antibodies to group *H. pylori* antigens (Epidemiol Infect 1997,119:29-34). We now examined these same 911 sera for antibodies to the CagA antigen of *H. pylori*. *H. pylori* strains may be *cag*$^+$ or *cag*$^-$, and serological responses to the CagA protein detect carriage of *cag*$^+$ strains. Paired serum samples collected in 1973 and 1994 from 221 subjects, also were studied. As expected, the CagA$^+$ seroprevalence fell from 36.5% in 1973 to 20.4% in 1994 ($p<0.001$). Over the 21-year interval, the greatest change was among persons <45 years. In this group, the decline in carriage of *cag*$^+$ *H. pylori* strains (34.3% to 8.0%) was significantly ($p<0.001$) greater than for cag$^-$ (11.6% to 5.9%) *H. pylori* strains. Of the 221 persons with paired serum samples, 35 showed changes in one or more assay, but only 20 (9.4%) actually changed *H. pylori* status. The estimated sero-conversion rate was 0.6 % per year and sero-reversion rate was 0.2% per year. With the exception of the few individuals who changed serostatus, the absolute levels of serum IgA and IgG to *H. pylori* and IgG to CagA changed little over the 21-year period.

Conclusions. We conclude that the decline in *H. pylori* prevalence during these 21 years predominantly affected *cag*$^+$ strains. Spontaneous loss of *H. pylori* appears to be uncommon in adults, whereas the estimated acquisition rate confirms that transmission in developed country populations does not exclusively occur during childhood.

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## Genetic Complementation of a Urease-Negative Helicobacter Pylori Mutant

HG1

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**Objectives:** In the last years several studies with urease-negative *H. pylori* mutants were undertaken. Irrespective of the studies' aims complemented mutants could serve as useful controls. Therefore we complemented the isogenic mutant N6*ureB*::TnKm originally derived from the parental strain N6 by shuttle mutagenesis (Ferrero et al).
**Methods:** Mutant N6*ureB*::TnKm carries a mini-Tn*3*-kanamycin transposon in the gene *ureB* resulting in a urease-negative phenotyp, no UreB and reduced UreA protein production. A 2.8 kb fragment including the genes *ureAB* as well as the promotor region located upstream of *ureA* was amplified by PCR using the plasmid pHP808 (Hu et al) as template and primers of 36 and 37 bp length, respectively. A *Bgl*II-site was integrated at the 5`-terminus of each primer. The PCR-product and the shuttle vector pHel2 (Heuermann et al) were digested with *Bgl*II. After dephosphorylation of pHel2, ligation was performed. Calcium-competent *E.coli* DH5α was transformed with the recombinant vector. In 10 clones selected with chloramphenicol the orientation of insert and resistence gene ($cat_{GC}$) to each other was assessed by digesting isolated plasmid with *Bam*HI. Plasmids of both orientations were used seperately for transforming *H. pylori* N6*ureB*::TnKm by electroporation and bacteria were spread on Brucella agar with 5% FCS, chloramphenicol and kanamycin.
**Results:** The frequency of urease-positive clones differed with respect to the gene orientation of *ureAB* and $cat_{GC}$: 52/70 (⇨⇦) and 5/70 (⇨⇨). Due to the copy number of the vector most clones produced even more urease than strain N6. Whole protein extracts of clones and N6 subjected to SDS-PAGE were similar except in the amount of UreA or UreB produced.

**HG2** Observations on regulation of gene expression in *Helicobacter pylori*

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The genome sequence of *H. pylori* suggests that the potential for transcriptional regulation is limited: transcriptional regulator homologues are sparse, and only three RNA polymerase sigma factors appear to be present. Cellular functions which might be influenced by environmental conditions include motility, urease expression, ammonia assimilation, and expression of oxidative stress resistance proteins such as alkyl hydroperoxidase AhpC. We have therefore investigated expression of genes related to these functions, to gain insight into control of transcription in this organism.

Primer extension experiments with primers designed to map the transcriptional start of *ureA/B, glnA, nixA* and *ahpC* showed transcription initiation from locations downstream of a consensus –10 promoter sequence, typical of the –10 region of housekeeping ($\sigma^{70}$) promoters in other organisms. In some cases, an extended –10 region with an upstream TG motif was also seen. No consensus sequence could be detected in the –35 region, unlike that of *C. jejuni* reported by Wostens *et al*. These observations confirm and extend those of Forsyth and Cover (1999).

To clarify the role of alternative sigma factors, $\sigma^{54}$ and $\sigma^{28}$, in *H. pylori*, we have constructed knock-out mutants in both the *fliA* and *rpoN* genes. Neither mutation was lethal, and the organisms were unaffected in their growth in the laboratory. Both mutants were however non-motile and did not express flagella, although some residual expression of flagellin(s) was apparent in both mutants. In confirmation of the lack of influence of RpoN on urease expression, this was normal in both mutants. Furthermore, 2D protein electrophoresis indicated that reduced levels of flagellin and FlgE expression were the only major alterations detectable in the knock-out mutants.

We suggest that control of transcription by differential promoter structure and alternative sigma factors is not prominent in virulence-related functions in *H. pylori*.

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**HG3** **The role of the Fur protein in regulation of *Helicobacter pylori* iron uptake**

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**Introduction**. Growth of *Helicobacter pylori* under iron-restricted conditions induces the expression of various proteins. The *H. pylori* genome encodes several homologs of iron uptake systems, but the regulation of these genes has not been studied.

**Aim**. Determine the role of the *H. pylori* Ferric Uptake Regulator (Fur) homolog in regulation of putative *H. pylori* iron uptake systems.

**Methods.** We selected five genes from the *H. pylori* 26695 genome sequence encoding homologs of iron-uptake systems: the *fecA* homologs Hp0807 and Hp1400, the *frpB* homologs Hp0876 and Hp0916, and the *feoB* homolog Hp0687. Transcriptional fusions of a *lacZ* reporter gene with these five genes were introduced into *H. pylori* 1061 (wild type) and its *fur* mutant derivative. Expression of the *lacZ* reporter was quantified using a β-galactosidase assay.

**Results.** Expression of three iron-uptake homologs (*frpB* Hp0916, *fecA* Hp1400 and *feoB* Hp0687) was constitutive in wildtype *H. pylori*, and not affected by the *fur* mutation. In contrast, expression of the two other iron uptake homologs (*fecA* homolog Hp0807 and *frpB* homolog Hp0876) was clearly Fur-repressed in wildtype *H. pylori*, as expression was 10-fold higher in the *H. pylori fur* mutant. Fur- and iron-regulation of *fecA* Hp0807 and *frpB* Hp0876 at the mRNA level was confirmed by Northern hybridization.

**Conclusions.** Two *H. pylori* putative iron-uptake systems are regulated by Fur, whereas three other putative iron-uptake systems are not. This suggests that in *H. pylori* these genes might not be iron-regulated. We are currently assessing the effect of metals and other environmental factors on the expression of these five genes.

## A human gene in the *H. pylori* genome: horizontal gene transfer from the eukaryotic host to a prokaryote. HG4

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A novel semi-automated computational approach was developed to search for genes present in bacterial genomes that have a eukaryotic origin. By using this tool a gene was identified in the *H. pylori* genome, HP0214, that bears closer resemblance to human and other mammalian homologues than to the bacterial orthologue. Several stringent screens were applied to confer the eukaryotic nature of the gene before a phylogenetic tree was constructed. The gene HP0214 is clearly grouped with mammalian genes and not with bacterial genes.
HP0214 encodes a $Na^+$ dependent transporter which is a transmembrane protein belonging to the Pho87 family. The substrate of the transporter is not known. Computational methods were applied to investigate and compare the properties of the predicted gene product to the human homologue protein. The presence of a mammalian-type transporter protein on the surface of *H. pylori* and its possible role in the process of adaptation to the host is discussed.

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Identification of a *Helicobacter pylori* strain with unique properties for intra- and interspecies plasmid transfer HG5

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*H. pylori* cells are naturally competent for uptake of both plasmid and chromosomal DNA. We asked whether there might be restriction barriers to transformation with plasmid DNA. *H. pylori* strain HPK5 was transformed to Kan$^r$ by electroporation with pHP1, a hybrid *H. pylori-E. coli* shuttle plasmid that carries a Kan$^r$ (*aph1*) cassette. pHP1$^{HPK5}$ then was isolated, and transformation of 31 other *H. pylori* strains with pHP1$^{HPK5}$ was attempted. pHP1 isolates from transformed strains were: (i) used to transform other *H. pylori* strains and *E. coli* DH5α and (ii), subjected to restriction endonuclease (RE) digestion and patterns compared. We found that each pHP1 isolate from a transformed *H. pylori* strain subsequently transformed its homologous strain better than heterologous strains. In total, 10 of the 32 *H. pylori* strains were transformed with pHP1 that was harvested from an *H. pylori* strain, but their RE profiles were generally different. pHP1 from strain JP26 (pHP1$^{JP26}$) was an inefficient donor, unable to transform any other *H. pylori* strain. Conversely strain JP26, the most transformable *H. pylori* strain studied, could be transformed by pHP1 derived from 7 other strains. 6 of 10 pHP1 derivatives could transform *E. coli* DH5α; pHP1$^{JP26}$ transformed DH5α at least 10-times as efficiently as pHP1 derived from the other *H. pylori* strains. As expected, all pHP1 isolates from DH5α, regardless of source, had identical RE profiles; however pHP1$^{(JP26\rightarrow DH5\alpha)}$ had a unique profile. These data suggest that DNA modifications in strain JP26 are unique among *H. pylori* strains, a property that may be used to facilitate interspecies genetic transfer with *E. coli*.

HG6 Purification of a novel endonuclease Hpy188I and cloning of its Restriction-Modification genes

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H. pylori are bacteria that colonize the human stomach. This colonization increases risk for peptic ulcer disease and gastric adenocarcinoma. Restriction-modification systems in H. pylori are not well-studied. We have isolated a novel restriction endonuclease, Hpy 188I, from H. pylori strain J188. Hpy 188I recognizes a unique specificity, TCNGA, and cleaves the DNA between nucleotides N and G in its recognition sequence to generate a one-base 3 overhang. Cloning and sequence analysis of the Hpy 188I modification gene in strain J188 reveal that hpy188IM has a 1134 bp ORF encoding a 377 aa product. The predicted protein sequence of M.Hpy188I contains nine conserved methylase motifs, which are arranged in an unusual pattern, compared to other methylases. Downstream of hpy188IM is a 513 bp ORF encoding a 170 aa product, that has a 41 bp overlap with hpy188IM. The predicted protein sequence from this ORF matches the amino acid sequence obtained from purified Hpy 188I endonuclease, indicating that it encodes Hpy 188I endonuclease. The Hpy 188I R-M genes are present only in three of twenty H. pylori strains tested. There is only one genotype at the hpy188IM-hpy188IR integrated region among H. pylori not carrying the Hpy188I R-M system. The significantly lower G + C content of Hpy 188I R-M genes implies that they had been relatively recently introduced into the H. pylori genome since the origin of H. pylori.

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HG7 Rapid, Directed Transposon Mutagenesis of *Helicobacter pylori*

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We have developed a method for rapidly generating stable, directed knockout mutants in *Helicobacter pylori* using a combination of *in vitro* transposition and natural transformation or mating. The gene of interest is amplified by PCR and cloned into a high-copy number plasmid such as pBluescript. The resulting plasmid is mutagenized *in vitro* using *Himar1* transposase and the transposon containing *aphA-3* (conferring kanamycin resistance) from *Campylobacter coli*. *Himar1* transposase recognizes the dinucleotide sequence TA for transposon insertion and is therefore capable of mutating essentially any gene. The mutagenized plasmids are repaired and replicated in *Escherichia coli* DH10B, purified, then introduced into *H. pylori* by natural transformation. Alternatively, a plasmid containing an origin of transfer sequence (oriT) has been transferred from *E. coli* SM10 by mating. Stable mutations are generated when the plasmids integrate into the *H. pylori* chromosome by double crossover. Mutants are recovered by plating on selective media containing kanamycin. This method eliminates the need for multiple PCR primers and cloning steps used in conventional knockout techniques. We have successfully used this method to mutate the *vacA* gene in *H. pylori* strain G27.

**Molecular Cloning and Characterization of *Helicobacter pylori* (*Hp*) 26695 *rfaJ* genes HP1416 and HP0159** HG8

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The lipopolysaccharide (LPS) of *Hp* is unique in that it contains the Lewis X (LeX) and/or Lewis Y (LeY) antigen epitopes mimicking those in the host; expression of these epitopes may promote escape from elimination by host immune system. In other bacteria, such as *Escherichia coli* and *Salmonella typhimurium*, *rfaJ* encodes α(1,2)-glucosyltransferase involved in the addition of distal glucose residues to the LPS core; however to our knowledge no α(1,2)-linked glucose has been found in *Hp* LPS. To determine the role of *rfaJ* in *Hp* LPS expression, we report the molecular cloning and characterization of two of the three recognized *Hp rfaJ* genes from 26695, designated HP1416 and HP0159. We constructed separate *rfaJ* 1416 and 0159 knockout mutants by insertional inactivation on the chromosome of 26695; the resultant strains were assessed for the expression of LeX and/or LeY by immunoblotting. Disruption of *rfaJ* 1416 did not affect the production of either antigen, whereas disruption of *rfaJ* 0159 abolished the expression of both Lewis epitopes. The fact that neither of the Lewis antigens are expressed in the *rfaJ* 0159 mutant suggests that either the individual monosaccharides that make up the LPS are not linked together and therefore the Lewis epitopes can not be attached, or the LPS is synthesized but attachment is impeded due to a defect in the LPS outer core. While not much is known about the LPS structure of *Hp* 26695, in other *Hp* strains N-acetyllactosaminoglycan (LacNAc) is a common component of LPS; genes encoding enzymes that synthesize this structure were not found in strain 26695. Since inactivation of *rfaJ* 0159 abolished expression of the Lewis epitopes, we hypothesize that this gene encodes one of the enzymes required for the synthesis of LacNAc.

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pH-Dependent Expression of Proteins in *Helicobacter pylori*, Identified by Two-Dimensional Gel Electrophoresis. HG9

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*H. pylori* survives transient exposure to extreme acid prior to adherence and growth on the gastric epithelium at neutral pH. The induction of protein expression by acid or base was tested in *H. pylori* 26695. Growth of *H. pylori* was attempted at a range of pH values from pH 4.5 – 8.9, in HPTY medium (2.5% tryptone, 0.75% yeast extract, 3% fetal calf serum, metal salts) with organic sulfonate buffers (100 mM) of appropriate $pK_a$ to maintain external pH during microaerobic growth. The range of growth observed was pH 5.6 – 7.5, with optimal growth rate at pH 6.5. Growth in acid resulted in a rise in pH, presumably mediated by urease. Growth in base, however, resulted in acidification. The effect of pH on growth suggests that metabolic pathways are regulated strongly by pH. To identify pH-dependent proteins, two-dimensional gel electrophoresis (2-D gels) was performed to separate whole-cell proteins of cultures grown to late log phase at pH 5.6-6.1 and pH 7.3-7.5. The gel conditions separated proteins within a range of pI = 4.5 – 6.5, and a range of molecular weight 10,000-70,000. The sequence of seven proteins was determined by N-terminal peptide analysis and compared with the *H. pylori* genome. Under all conditions, the major proteins present were the urease structural subunit UreB and GroEL. UreB expression was acid-inducible, with highest levels at pH 5.8. A protein induced over 25-fold by acid was tentatively identified as the ClpP ATP-dependent protease. A protein whose gel position was consistent with WbcJ (O-antigen biosynthesis) was induced 8-fold by acid. TsaA (26-kDa antigen), a homologue of the anti-oxidant AhpC, was induced by growth above pH 7. The pH dependence of specific proteins may enable *H. pylori* to survive exposure to extreme acid.

**HG10** Transposon Shuttle Mutagenesis of *Helicobacter pylori* Strain 26695

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**NOTES**

The chromosomes of *H. pylori* strains 26695 and J99 have been completely sequenced; each strain has about 7% unique genes. Since about one-third of the *H. pylori* genes are of unknown function, it is important to develop tools to investigate functions of unknown genes. Construction of a library of mutants which could then be screened for various phenotypes *in vitro* or *in vivo* is one approach to ascribe functions to unknown genes. We have constructed such a library in strain 26695 using transposon shuttle mutagenesis. This method was previously used by Rainer Haas and colleagues to construct mutants in *H. pylori* strains P1 and P12; this method, however, has not been applied to either of the two strains whose genome sequence is known. For transposon shuttle mutagenesis, a genomic library of strain 26695 was cloned into plasmid pHSS5CatΔKan, which is susceptible to transposition by *mTn3*-kan, a defective transposon. The *mTn3*-kan was incorporated onto an F' derivative for conjugative mobilization into *E. coli* strains containing transposase and resolvase so that transposition of *mTn3*-kan into the pHSS5CatΔKan-library and resolution of the resulting cointegrate could be controlled. Restriction enzyme analysis of plasmids revealed that transposition occurred randomly but only once per plasmid. Mutagenized plasmid pools were isolated and electroporated into strain 26695. Southern blot analysis of chromosomal DNA from 23 of over 900 kanamycin-resistant mutants recovered revealed that at least 60% of the mutants were unique and that there was only one copy of the defective transposon per mutant. These results indicate that we have successfully obtained transposon mutants of *H. pylori* strain 26695.

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**HG11** Iron-sulfur cluster assembly in *Helicobacter pylori*

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The *H. pylori* genome sequence predicts over a dozen proteins which contain iron-sulfur clusters. These proteins serve a variety oxidation-reduction (electron transfer) and catalytic roles. Although it is not well understood how iron-sulfur clusters are assembled *in vivo*, two proteins (NifU and NifS) implicated in the process are present in the *H. pylori* genome. In the best-studied example to date, *Azotobacter vinelandii*, NifU is thought to donate the iron and NifS the sulfur to the cluster. Attempts to obtain mutants in genes encoding these proteins (TIGR #'s HP0220 and HP0221) have been unsuccessful, despite the fact that five separate *H. pylori* genes were disrupted at high efficiency in parallel transformations. That NifU and NifS are required for the survival of *H. pylori* is not surprising due the importance of various iron-sulfur cluster containing proteins in basic metabolic processes. We have now turned to an *in-vitro* approach to studying the (purified) proteins following their over-expression in *Escherichia coli*. NifU contains the conserved cysteine residues which ligate an iron-sulfur cluster as well as the free iron that is donated to newly formed clusters. Purified NifU has the characteristic reddish-brown color and spectral characteristics of a 2Fe-2S cluster. UV-Vis, EPR, and MCD studies confirm the presence of the cluster. Partially purified NifS has spectral characteristics indicating the possible presence pyridoxal-phophate prosthetic group: such a prosthetic group is proposed for the *A. vinelandii* enzyme.

## *hypA* gene in *Helicobacter pylori* HG12

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*Helicobacter pylori* expresses a 'hydrogen – uptake' type hydrogenase. Hydrogenase activity is readily detectable in whole cells with oxygen as terminal electron acceptor. Although hydrogenase activity is constitutive, the activity is reduced upon incubation of cells in an atmosphere lacking hydrogen. No difference in growth rate was observed for cells growing in the presence or absence of hydrogen. The *hyp* genes of the *H. pylori* genome encode accessory proteins, which are essential for the maturation of the hydrogenase enzyme. Inactivation of the *hypA* gene by kanamycin cassette insertion resulted in a mutant with negligible hydrogenase activity as compared to the wild type. Despite the lowered activity, expression of the hydrogenase enzyme seems to be unaffected as determined by the western blot analysis using antibodies against the large subunit of hydrogenase. The *hypA* mutant showed no difference in the growth rate when compared to the wild type, when grown in presence of 10% hydrogen or in an atmosphere lacking hydrogen, or in differing levels of oxygen.

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## The use of *lacZ* and *cat* as reporter genes in *Helicobacter pylori*: detection and quantification of the gene products with commercial ELISA kits HG13

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In order to evaluate whether the beta-galactosidase gene *lacZ* and the chloramphenicol-acetyltransferase gene *cat* can be used as reporter genes in *H. pylori* the genes were integrated in the chromosome and the gene products were quantified with commercial ELISA systems (Boehringer-Mannheim).

For integration of the *lacZ* marker gene into the chromosome, two new suizide plasmids designated as pLAC-KM and pLAC-CAT were constructed. Both vectors contain a unique *Hin*dIII site for cloning of *H. pylori* DNA fragments in front of a promoterless *E. coli lacZ* gene which is fused to a *neo* or a *cat* resistance gene, respectively. The plasmids were ligated with *H. pylori* DNA and transformed into *H. pylori*. Mutants which carried *lacZ* insertions in the chromosome were selected with kanamycin or chloramphenicol. The mutants expressed the *lacZ* gene as determined by screening with a *lacZ* color substrate assay. For testing of the *cat* gene, a promoterless version of this gene was inserted into the *H. pylori* ferritin gene *pfr* and the gene expression was analysed under various concentrations of iron and nickel. Measurements of the amount of LacZ and Cat enzymes by ELISA indicated that the assay systems were well suited for the detection and quantification of both reporter proteins in the mutants. The parental strains were negative in both assays. The upregulation of the ferritin gene by iron and its repression by iron starvation could be quantified with the Cat-ELISA system. The anaylsis revealed that iron overload induces *cat*-transcription at least five fold, wherase iron depletion and nickel overload reduced *pfr* expression to about 20 and 50 %, respectively.

In conclusion the genes lacZ are excellently suited for quantitative reporter gene analysis in *H. pylori*.

NOTES

**HG14** **Regulation of the *Helicobacter pylori* Ferritin-Gene *pfr* and the Role of Ferritin in Metal-Resistance**

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It has been observed earlier that the ferritin gene *pfr* of *Helicobacter pylori* is involved in iron-resistance and that the ferritin protein accumulates under conditions of iron overload. It was the aim to investigate whether the ferritin is involved in resistance to other metals and to determine whether the iron-mediated regulation of the ferritin occurs at the transcriptional level. A promoterless chloramphenicol-acetyl-transferase (*cat*) cassette inserted in the *pfr* gene of *H. pylori* strain G27 was used as a reporter for transcriptional activity of the *pfr* gene. Production of the ferritin and of the Cat protein was monitored by Westernblot. The iron content of bacteria was determined by using a kit for detection of iron in human sera. Resistance to metals was determined in Brucella broth with 10 % fetal calf serum (BBF) supplemented with metal-chlorides. Analysis of Cat production in the *pfr* mutant revealed that growth in presence of iron induces the *pfr::cat* fusion. The upregulation was specific for iron as production of Cat and of ferritin was not induced by other metals like Mn, Cu, Ni, or Zn. A *feoB* mutant of *H. pylori*, in which uptake of ferrous iron is impaired, showed deregulated production of the ferritin. The ferritin-mutant contained less iron than the parental strain and showed an increased sensibility to cobalt which was found to be toxic for *H. pylori* in the range of 40 µM. Bismut was also toxic at low concentrations (20 µM) but the ferritin was not involved in resistance to this metal. The results indicate that iron-dependent induction of ferritin synthesis occurs on the transcriptional level. The lowered total iron content of the *pfr* mutant supports the role of ferritin in storage of iron and provides evidence for a link between ferritin activity and iron-dependent regulation of iron uptake. The sensitivity of the ferritin mutant to cobalt indicates that metal-resistance mediated by ferritin is not specific for iron.

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**HG15** **Metal-dependent Regulation of Ferritin (Pfr) Synthesis in *Helicobacter pylori*: Induction by Iron of strain-specific Isoforms and Repression by the Ferric Uptake Regulator in Reponse to Iron Starvation**

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The iron-dependent ferric uptake regulator protein Fur downregulates genes involved in iron uptake. To determine the role of Fur in metal-dependent regulation of *H. pylori* genes, a *fur*-deficient mutant of strain NCTC11638 was constructed by marker exchange mutagenesis. Growth experiments were performed in BBF liquid medium supplemented with chloride salts of Fe, Ni, Cu, Zn, or Mn. Iron restriction was created with the iron chelator desferal. The Pfr protein was detected by Westernblot with the polyclonal antiserum AK198. 2D-PAGE was performed with a IGPphor system (Pharmacia). Comparative analysis of protein producion in the parental strain and in the *fur* mutant grown under the various conditions revealed that synthesis of the ferritin protein is differentially regulated in the *fur* mutant. In both strains (wt and fur-negative mutant) iron overload induced the formation of five Pfr isoforms. Analysis by 2D-PAGE showed that the isoforms differ in molecular weight and in the isoelectric point. Furthermore, Pfr production was repressed by nickel, copper, zinc, and manganese in the parental strain but not in the *fur* mutant. Surprisingly, iron depletion also strongly inhibited ferritin biosynthesis in the parental strain but not in the *fur* mutant which overproduced ferritin even under normal conditions. The function of Fur in regulation of ferritin biosynthesis including responses to iron starvation and metal overload indicates that Fur does play an important role in overall metal-dependent regulation. The derepression of ferritin production in the *fur* mutant under conditions of iron starvation indicates that Fur can be involved in regulation processes also if the iron concentration is low. This regulatory activity of Fur in *H. pylori* gives new insights into Fur function as comparable regulatory function has so far not been investigated in other bacteria.

HG16

**Identification of Fur-regulated promoters of *Helicobacter pylori* by Adaptation of the Fur Titration Assay**

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The Fur titration assay (FURTA) is a powerful technique for identification of genes regulated by the ferric uptake regulator protein Fur. The assay is based on Fur-mediated changes in expression of an *E. coli lacZ* reporter gene which are easy to detect. Because the original assay with *E. coli* Fur was not suited for identification of Fur-regulated genes of *H. pylori*, the FURTA was adapted for analysis of *H. pylori* DNA by production of the *H. pylori* Fur protein in a *fur*-negative indicator strain of *E. coli*. The *fur* gene of *H. pylori* was transferred in the *fur*-negative *E. coli* strain H1780 (*fiu::lacZ*), resulting in strain H1780-Hp. Production of *H. pylori* Fur restored iron-dependent regulation of the reporter gene. The FURTA was performed by reading the colour of colonies of H1780-Hp on McConkey agar supplemented with ferric chloride. A FURTA-positive phenotype was indicated by a red color of H1780-Hp in presence of iron. Plasmids from a DNA library, which mediated a FURTA-positive phenotype, carried an identical 1 kb insert containing the promoter region and the N-terminal part of a *H. pylori fecA* homolog (HP0807). This DNA was negative in the original FURTA with *E. coli* Fur. Separate analysis of subcloned promoters of HP0807 and in addition of the *ribBA* gene, which was shown earlier to be regulated by iron, confirmed the FURTA-positive phenotype of the HP0807 promoter, and indicated that Fur interacts with the *ribBA* promoter. The production of the *H. pylori* Fur protein in an Fur-indicator strain of *E. coli* adapted the FURTA for analysis of *H. pylori*-DNA which does not interact with *E. coli*-Fur. The iron-dependent regulation of the *fecA* homolog (HP0807) is now being confirmed on the mRNA level.

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HG17

**Simultaneous analysis of *Helicobacter pylori* mutants using unique DNA tags and a high-density oligonucleotide array**

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Labelling bacterial mutants with unique DNA identifying sequences, commonly referred to as DNA tags, was first performed using transposons (signature tagged mutagenesis, STM). However, the usefulness of STM is reduced by the requirement for transposons, their random insertion in the genome and the limitations of the filter-based hybridisation assay. Defined bacterial mutants can be constructed using Inverse PCR Mutagenesis (IPCRM), which is well established for *H. pylori*. Using a modification of the IPCRM methodology, a unique DNA tag can be incorporated upstream of a defined deletion and selectable marker in any cloned *H. pylori* gene. These constructs are used to produce tagged defined *H. pylori* mutants by homologous recombination-mediated allelic replacement. Through oligonucleotide design, the unique DNA tags can be detected by hybridisation to a high-density oligonucleotide array containing tag complements. This allows mutants to be studied *en masse* in both *in vitro* and *in vivo* assays. We have termed this procedure Signature Tagged Allelic Replacement (STAR).

Primers were designed to amplify over 50 genes, selected from the *H. pylori* 26695 genome sequence. The genes were amplified by PCR, cloned into pUC18, and used to construct defined tagged *H. pylori* mutants by STAR. Analysis has shown that the mutants can be grown as a pool in liquid culture and individually detected using the high-density oligonucleotide array. A group of tagged mutants was used to study the growth of pooled *H. pylori* mutants under the *in vitro* stress conditions of low pH and low iron concentrations. Currently, these mutants are been used to apply this technique to *in vivo* analysis through the colonisation of *H. pylori* animal models.

**HG18** **Identification and characterization in *H. pylori* of NikR an homologue of a nickel-responsive regulator in *Escherichia coli***

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Analysis of the two HP genomes revealed the presence of a gene encoding an homologue of NikR, a nickel-responsive regulator which represses the nickel-specific transport *nik* operon in *E. coli.*
A *nikR* deficient *H. pylori* mutant of SS1, was constructed by replacing the *nikR* gene with a non polar kanamycin cassette. Rabbit antibodies raised against the protein confirmed the absence of expression of NikR in the mutant. Protein analysis of the parental and the mutant strains demonstrated the overexpression of several proteins within the mutant strain, suggesting a regulatory function for NikR in *H. pylori*. NikR had no direct effect on the expression of UreA, UreB or HspB. In order to identify the gene regulated by NikR, a library of random fragments (1-kb in size) was constructed within a vector in which i) *nikR* was cloned under the control of a *ptac* promoter and ii) a ß-galactosidase reporter gene was introduced downstream of transcriptional stops. The library contained in 107 565 clones. 1321 clones were ß-galactosidase positive in the absence of IPTG, and 30 of them demonstrated a reduced ß-galactosidase activity when grown in the presence of IPTG. We are currently determining the identity of these genes. In addition, NikR of *H.pylori* was unable to complement the NikR function of *E. coli.*

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**HG19** **Systematic Cloning of the 1590 Orfs of the 26695 Genome as a Tool for *H. pylori (Hp)* Gene Expression and Functional Analysis**

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Forward and reverse oligonucleotides of the 1590 open reading frames (ORFs) of the genome of *Hp* strain 26695 were designed in order to allow full length amplification of each of the ORFs. Every forward and reverse oligonucleotide was tagged respectively at its 5'extremity with a CAUCAUCAU and a CUACUACUA sequence. Genomic DNA of *Hp* strain 26695 was used as a template for PCR. Seventeen times 96 amplicons were cloned in parallel after an Uracil DNA Glycosylase (UDG) treatment of both the amplicon and the vector (3.6 kb, $Sp^R$) which resulted in the production of 3' protruding ends on both. Annealing was completed at 37°C for 30 mn in 96-well plates producing chimeric molecules that were transformed in *E. coli* without the addition of ligase. Enrichment of transformants was conducted in microtiter plates in selective liquid medium and the final selection of individual transformants was obtained by spotting 10 µl of the 96-well liquid selection on solid selective medium plates. This procedure resulted in the fast and efficient cloning of more than 80% of the orfs of the whole genome. The cloning efficacy was independent of the size of the amplicon when ranging from 200 bp to 3 kb. Larger orfs were cloned as two amplicons. *E. coli* clones harboring the recombinant plasmids were stored at -80°C and will be processed for various applications: 1) the recombinant plasmids were transformed into HB101(pTCA), a strain encoding the Tn*3* transposase; this will allow to conduct in parallel the systematic disruption by miniTnKm of all the genome orfs in *E. coli,* and ultimately to test individually any gene of interest to assess its requirement for in vitro and/or in vivo multiplication (see P. Jenks'communication). 2) to serve as DNA driver for gene expression studies, as well as for studying the genomic diversity among clinical isolates, and 3) ultimately, to compare the benefit of using such recombinant clones rather than amplicons as drivers for the gridding of filters.

## Characterization of the two paralogous aliphatic amidases of *Helicobacter pylori* HG20

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Genome sequences of *H. pylori* have permitted to classify a majority of genes into orthologous and paralogous families. Orthologs are genes related by vertical descent after speciation events, whereas paralogs are homologs generated by duplication and which have diverged sufficiently to achieve similar but not identical functions. The present study was undertaken to characterize two paralogous genes, *amiE* (HP 294) and *amiF* (HP 1238), which encode two aliphatic amidases (EC 3.5.1.4). As urease, these two enzymes (which present 34% sequence identity) hydrolyse amide groups to produce ammonia which plays a key role in the *H. pylori* pathogenicity. The *amiE* gene was cloned from a cosmidic library of *H. pylori* strain 85P, and *amiF* by PCR amplification from the sequenced strain 26695. The two genes were overexpressed in *E. coli*, and the two enzymes were purified as follows: first with a strong anion-exchange carrier and then, fractions carrying amidase activity, were submitted to a gel filtration step. The results revealed that these two proteins: (i) both possess homotetrameric structures under native conditions, (ii) have distinct biochemical properties: optimal AmiE activity was observed at pH 7.0 and at 55°C, whereas optimal activity for AmiF was measured at pH 6.0 and at 45°C, and (iii) present distinct substrate specificities: AmiE degrades propionamide, acetamide, acrylamide (as the other aliphatic amidases described from *P. aeruginosa* and *Rhodococcus sp.*R312), whereas AmiF only hydrolyses formamide. AmiF is thus the first aliphatic amidase described to be specific for a single substrate. Additionaly, *H. pylori* mutants deficient in *amiE* and *amiF* were constructed by allelic exchange in strain N6. *In vitro* assays, performed on sonicated extracts, revealed that *amiE* is well expressed and its synthesis increased in the absence of urease. In contrast, *amiF* is poorly expressed. The role of these two proteins in *H. pylori* metabolism and the *amiE / amiF* distribution in different *H. pylori* strains are presently investigated.

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## Detection of *Helicobacter* specific sequences in European farm foxes HG21

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The occurrence of *Helicobacter*-like organisms (HLOs) in farm foxes has already been described. Whether these bacteria belong to the genus *Helicobacter* is as yet unknown. **Material and methods**: We therefore investigated samples of gastric tissue (antrum and corpus) from 6 silver foxes (*Vulpes vulpes*) and 4 blue foxes (*Alopex lagopus*) by using histopathological, immunohistochemical and electronmicroscopical methods. DNA of the samples was extracted and a nested PCR was applied using the product of a bacterial universal 16S rRNA gene amplification as template for a *Helicobacter*-genus specific PCR. Products of this second reaction were used for DNA sequencing. **Results**: By microscopical methods HLOs with up to 8 loose spiral turns were demonstrated in the gastric mucosa of 9 foxes. Their morphological appearance was similar to that of *H. heilmannii*. In all foxes colonized with HLOs, a cellular reaction in the form of focal accumulations of lymphocytes in the gastric mucosa was found by histological examination. Lymphoid follicles were found in 4 foxes. Macroscopical lesions were not detected. Two dominant HLOs were found to be present in farm foxes and tentatively identified, by 16S rDNA alignment, to be *Gastrospirillum hominis* (clone G2A9) and *Helicobacter* sp. (UNSW1.7sp). Only one of these types was found per individual animal and the same HLO colonised both antrum and corpus. There was no linkage between fox species and HLOs present. **Conclusions**: HLOs were found in both fox species and, in some cases, associated with a histopathological response. Preliminary sequence analysis of the 16S rDNA has shown that these bacteria belong to the genus *Helicobacter*. However, sequence similarities between 90% and 99% would indicate that some of the fox HLOs may be new *Helicobacter* species.

HP1

## A CAT MODEL OF GASTRIC DISEASES ASSOCIATED WITH HELICOBACTER FELIS (H. FELIS) INFECTION

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Chronic H. pylori infection is a focus of clinical interest with respect to pathogenesis of chronic gastritis and duodenal ulcer that may further serve as the pathogenic basis for development of tumors. In the present study, to investigate the pathogenic mechanism underlying development of gastrointestinal diseases, we tried to establish a cat model of gastrointestinal ulcer by infection with H. felis, a species related to H. pylori.

As demonstrated by colony formation assay, viable H. felis cells were recovered from 9 of 12(75%) samples of gastric mucosal membranes collected from cats in which H. felis was introduced into the stomach, indicating that the introduced H. felis successfully established itself in the stomachs of these cats. Upon autopsy, duodenal tissues from 6 cats(50%) macroscopically showed a light degree of inflammation and ulcer-like lesions. Histopathological examination of stomach tissues obtained from cats with duodenal ulcer-like lesions revealed swollen mucosal cells, ablation(localized erosion), neutrophil infiltration into epithelial cells, and expansion of crypts in these lesions. In addition, macrophage and neutrophil infiltration were observed in propria flanked by epithelial degeneration and necrosis.

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HP2

## Urease is required for intestinal colonization of mice by *Helicobacter hepaticus*.

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We have inserted a chloramphenicol resistance cassette into the *ureB* gene of *H. hepaticus*. Using high voltage electroporation, allelic exchange was performed to generate isogenic mutant strains that had no detectable urease activity. Urease mutants had no obvious growth defect in vitro. However, the mutants did exhibit reduced viability at low pH in the presence of urea when compared to the wild type parental strain. Outbred Swiss Webster mice were orally inoculated with urease mutant strains or with the wild type parental strain of *H. hepaticus*. By 1 week post-inoculation, *H. hepaticus* could not be detected in the feces of mice inoculated with urease mutant strains using PCR. Failure of the urease mutant strains to colonize was confirmed by culture and by a specific fecal IgA ELISA. Mice inoculated with the wild type parental strain were persistently infected as judged by PCR, culture, and fecal IgA ELISA. Allelic exchange in *H. hepaticus* has now been successfully performed. Urease appears to be required for intestinal colonization of laboratory mice by *H. hepaticus*. The exact role of urease activity in intestinal colonization remains to be determined.

HP3

Reliability of detection of Helicobacter pylori in feces in eradicated and non eradicated patients.

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Non invasive testing for the detection of Helicobacter pylori (Hp) is presently based mainly on serology and Urea Breath Test (UBT). Both methods suffer some limitations (unreliability of serology in eradicated patients, high costs of equipment of UBT). Recently, the detection of Hp in feces has been proposed as a reliable test at reasonable cost. However, only a limited amount of data is available, and a high rate of false positive results has been recently reported in eradicated patients

Aim of this study was to evaluate the sensitivity and specificity of the detection of Hp in stools both in eradicated and non eradicated patients.

Patient population was made of 77 patients, 62 untreated and 15 submitted to the evaluation at least 2 months after Hp eradication for dyspeptic symptoms. All patients underwent endoscopic assessment of Hp infection by histology and culture. Patients were considered infected when culture and/or histology gave a positive result, whereas when both patients tested negative ,the patient was considered not infected. Fecal testing showed the following values: sensitivity of 84% and specificity of 88%. Among patients in whom eradication was attempted in the past, the test showed a specificity of 85% and a NPV of 92%. Sensitivity and PPV were not calculated since the only Hp positive patient was negative at fecal test.

These data suggest that fecal testing for Hp is reliable both in eradicated and non eradicated patients. In particular, in contrast with recent data, we did not find false positive results. However more data are needed in patients in whom eradication failed for the evaluation of the testsin this context.

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HP4

Endoscopic evaluation of *Helicobacter pylori* in clinical practice.

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Available data on sensitivity and specificity of culture, histology and rapid urease test (RUT) for the diagnosis of *Helicobacter pylori* (Hp) infection may not correspond to those obtained in clinical practice for various reasons: as laboratory expertise, kind of endoscopic population, and concomitant therapy. In order to obtain this information, we performed a 2 year nationwide survey regarding 15 Italian Centers. The number of patients tested for Hp, the type of test, the endoscopic finding and information about past attempted eradication were recorded by each center. Patients with peptic ulcer probably due to NSAIDs were excluded. For the purpose of this study we assumed 100% rate of Hp infection for patients with peptic ulcer disease (PUD) and no false positive results for culture or histology. As far as RUT sensitivity and specificity in patients with normal endoscopy (NE) and in those with past attempted eradication are concerned, we evaluated patients who performed concomitantly culture, histology and RUT and we considered as positive those in whom culture or histology were positive, and as negative those in whom culture and histology were negative. 1048 patients were tested for Hp, 653 of them were concomitantly evaluated with the 3 tests. According with our criteria, among 80 patients with PUD RUT showed 83% of sensitivity and 91% of specificity, the positive and negative predictive value were respectively98% and 91%. Among 300 patients with NE, RUT was 70% sensitive, 97% specific, its positive predictive value was 98% whereas its negative preditctive value was 65%. Among 125 patients with post attempted therapy the sensitivity of RUT was 48%, its specificity was 100%, ist positive and negative predictive value were respectively 100% and 78%.These data indicate that the sensitivity of invasive tests for diagnosis of Hp infection in clinical practice is lower than that reported in research studies and that a negative RUT test in patients with normal endoscopy or with a prevoious eradication therapy cannot be considered reliable.

NOTES

HP5 **The Fur homologue of *Helicobacter pylori* is involved in acid-resistance**

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**Objective.** Apart from its role in iron acquisition, the ferric uptake regulator protein (Fur) of *Salmonella typhimurium* is also involved in acid-resistance. The aim of this study was to investigate whether the recently identified Fur homologue of *Helicobacter pylori* also has a role in acid-resistance.
**Methods.** A *H.pylori fur* mutant was created in strain 1061 by allelic exchange mutagenesis. The growth of mutant and parental strain was assayed qualitatively on plates with a pH ranging from 4.5 to 7 and quantitatively in broth with a pH of 5.3 and pH 7, either with or without addition of 5 mM urea. Bacteria grown in broth were analyzed by SDS-PAGE gel electrophoresis and total protein profiles were compared.
**Results.** At low pH growth of the *fur* mutant was severely impaired compared to the parental strain. However, at pH 7 and when 5 mM urea was added to the pH plates, the growth of the mutant and the parental strain was identical. Interestingly, a 20 kDa protein was overexpressed in the Fur mutant at both pH's.
**Conclusion.** The fact that addition of urea restores the ability of the Fur mutant to grow at low pH shows that the urease dependent acid-resistance is fully functional in this mutant. Therefore, we conclude that the Fur homologue of *H.pylori* plays a substantial role in the urease independent acid-resistance of *H.pylori.*

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HP6 ***In Vivo*-Distribution of *Helicobacter felis* in the Gastric Mucus of the Mouse**

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A method is described that permits collection of very small samples (2 nl) from precisely defined positions within the gastric mucus of anesthetized mice. This method was used to study the *in vivo* local distribution of bacteria within the mucus of *Helicobacter felis*-infected mice. Each sample was ejected onto a microscope slide and microscopically analyzed, within less than 1 min, as a native preparation. To avoid changes in bacteria location within the mucus after collection, bacterial motility was blocked by adjusting the pH inside the collecting pipette to 4.5.
The mucus of the collected sample was subdivided into three layers, an epithelial layer (the first 25 µm mucus from the tissue/mucus surface), a luminal layer (the last 25 µm to the mucus/lumen surface) and the remaining central mucus layer.
The volume of the analyzed segments in the sample was between 4 and 9 picoliters. The concentration of bacteria inside the epithelial mucus layer was 3,400 per nl, but was only 50 per nl inside the central and luminal mucus layer. The mean distance of *H. felis* to the epithelial surface was 16 µm. A total of 75% of all *H. felis* bacteria resided in the mucus zone between 5 and 20 µm from the tissue surface, with no bacteria closer than 5 µm to the epithelial surface. This method permits the study of factors determining the density of colonization and distribution of bacteria along chemical gradients with high precision.

HP7

*Helicobacter*-induced expression of Bcl-xL in B-lymphocytes. Apoptosis inhibition as an important step in the development of gastric MALT lymphoma

A. MORGNER, J.L. O'ROURKE, P. SUTTON, A. ENNO, M.F. DIXON and A. LEE, UNSW Sydney, Australia, University of Leeds, Leeds, U.K.

**Aim:** Primary gastric B-cell MALT lymphoma may develop from chronic infection with *Helicobacter sp.* To increase our understanding of this tumour we investigated a regulator of apoptosis, Bcl-x, in an animal model of MALT lymphoma. *Bcl-x* is a *bcl-2*-like gene which encodes two transcripts. The longer one codes for *bcl-xL* which protects cells from apoptosis, whereas the shorter *bcl-xS* transcript accelerates death. **Methods:** We investigated the in vivo distribution of bcl-xL in MALT lymphoma by immunohistochemistry and *bcl-xL* mRNA expression in B-lymphocytes from the spleen and stomach of short and long term infected mice. RT-PCR was performed with primers specific for both *bcl-xS* and *bcl-xL*. **Results:** Immunohistochemical analysis showed up to 75% of B-lymphocytes were positive for Bcl-xL in all MALT and low-grade MALT lymphomas where as high-grade MALT lymphomas showed no immunoreactivity for *bcl-xL*. RT-PCR analysis of *bcl-xL* mRNA expression from gastric B-lymphocytes of short and long term infected animals showed *bcl-xL*, but no *bcl-xS* expression as expected. Cultivated splenic B-cells did not express *bcl-xL* or *bcl-xS*, however, after B-cell stimulation with *Helicobacter* antigen, bcl-xL but not bcl-xS was expressed. **Conclusion:** *Helicobacter*-antigen induces expression of Bcl-xL in B-lymphocytes in vivo and in vitro, protects B-cells from apoptosis, and prolongs cell survival. In high-grade B-cell MALT lymphoma expression of Bcl-xL is downregulated which could be a sign of an antigen-independency. Bcl-xL may play a critical role in the pathogenesis of B-cell MALT lymphomas via an effective inhibition of apoptosis.

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HP8

Gastric B-cell MALT lymphoma and *Helicobacter heilmannii* infection

J.L. O'ROURKE, A. ENNO, M.F. DIXON, A. JACK and A. LEE. UNSW, Sydney, Australia & University of Leeds, Leeds, U.K.

**Background:** We have previously shown that long term infection of BALB/c mice with *Helicobacter heilmannii*-like organisms (HHLO's) resulted in animals exhibiting gastric B-cell MALT lymphoma. **Aim**: To determine if different HHLO's originating from a range of animals and human patients were able to induce different patterns of lymphoma development. **Methods:** A total of 294 animals were infected for 22-28 months with one of 10 different HHLO's and compared with groups of mice infected with *H. felis* and *H. pylori*. **Results:** A lymphocytic infiltrate was seen in all infected animals which in the majority of animals (83.6%) was characterised by the presence of lymphoepithelial lesions (LEL). In some cases this progressed to MALT lymphoma, as diagnosed by pathology. Low grade lymphoma (LGL), defined as the presence of a diffuse infiltrate of centrocyte-like cells which invaded the gastric epithelium resulting in destructive lymphoepithelial lesions was seen in 13.6% of infected animals. When large sheets of blast-like cells were observed high grade lymphoma was diagnosed (4.1% of infected animals). However, significant differences were observed in the prevalence of these features which was dependant upon the infecting organism. **Conclusion:** The high prevalence of MALT lymphoma seen in these animals corresponds with a report of a high prevalence of this malignancy in humans infected with *H. heilmannii*. High grade lymphoma was most commonly found in animals in which there was a high prevalence of LELs and LG lymphoma supporting the concept of transformation from low grade to high grade lymphoma. This model will now allow us to look for genetic and other changes involved in the induction of gastric MALT lymphoma.

**HP9** Coexistence of Helicobacter species in the one ecological niche – Coinfection of ferret gastric mucosa with *H. mustelae* and *H. heilmannii*-like organism.

J.L. O'ROURKE, K. ROSE, A. BLACKSHAW & A. LEE UNSW & Taronga Park Zoo, Sydney, & Royal Adelaide Hospital, Adelaide, Australia.

Gastric tissue from 14 normal ferrets, aged 2 – 30 months, was obtained from an experimental colony maintained in South Australia and examined for the presence of *Helicobacter mustelae*. Silver stained sections revealed the presence of *H. mustelae, which* was readily cultured from the gastric tissue. The bacteria heavily colonised the antral crypts but could also be found on the surface epithelium and upper regions of the corpus crypts. Surprisingly a second bacterium with morphology resembling *"Helicobacter heilmannii"* was also detected, a finding which has not been previously reported in the ferret. The two species of *Helicobacter* had very different distributions in the stomach indicating that they inhabit different ecological niches. Unlike *H. mustelae*, the ferret strain of *H. heilmannii* showed significant colonisation of the body mucosa with bacteria penetrating deep into the gastric crypts and occasionally within parietal cells. The pathology seen in the animals correlated with their age with minimal to mild infiltration in the young animals which progressed to a severe infiltration in the older animals. This first report of dual natural infection of ferrets provides interesting opportunities to experimentally manipulate the gastric environment and learn more about factors that determine why *Helicobacter* species colonise where they do. There is a continuing debate as to whether all the *H. heilmannii*-like organisms from different animals should be given a species name. We think this to be unnecessary but should a name be needed we suggest the name *"Helicobacter foxii"*!!

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**HP10** Urease and ureI form a membrane complex enabling selective periplasmic pH regulation

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*H. pylori* urease is a neutral pH optimum enzyme found both in the cytoplasm and on the outer surface of the organism. Cytoplasmic urease is activated when the $pH_{out}$ falls below 6.5 and remains active down to pH 2.5. We have recently shown that ureI is an acid activated urea transporter responsible for the increase in urease activity as $pH_{out}$ becomes acidic. We proposed that as $pH_{out}$ drops, $H^+$ and urea enter the periplasmic space resulting in a decrease in the periplasmic pH. This decrease in periplasmic pH results in a conformational change in the periplasmic loops of ureI allowing urea to enter the cell. In the cell interior, urea is rapidly hydrolysed by urease to $CO_2$ and $2NH_3$. The ammonia then diffuses into the periplasmic space and becomes protonated resulting in an increase in periplasmic pH. We have no evidence for any increase in cytoplasmic pH following urea addition suggesting interaction between the site of urea entry and urease activity. To further characterize the ureI protein we assayed purified membrane preparations of *H. pylori*, *H. pylori* urease mutants and *H. pylori* ureI mutants by native gel electrophoresis followed by Western blotting using antibodies to ureA, B and I. Magnetic bead immuno-precipitation using the same antibodies was also performed. Native gel Western blots of membrane preparation of *H. pylori* probed with ureI, ureA and ureB antibodies revealed a single band of >200kd. Similar blots of the ureI mutant failed to detect any bands. Magnetic bead immunoprecipitation using ureI antibodies co-precipitated both ureA and ureB. This result was confirmed by immunoprecipitation using ureA or ureB antibodies which co-precipitated both ureI and either ureB or ureA respectively. We conclude that ureI tightly binds a fraction of the urease in close apposition to the inner membrane allowing rapid diffusion of $NH_3$ into the periplasmic space resulting in an increase of periplasmic pH without disturbance of cytoplasmic pH.

NOTES

HP11

Gastric Homing of CD4+ cells in Recipient SCID Mice Varies Based upon Infection Status of the Donor. R.A. PETERSON, T. HOEPF and K.A.EATON; the Ohio State University Columbus, OH, 43210, USA.

**Objective:** To compare gastric CD4 cell influx in *H.pylori* infected and uninfected C57BL/6 mice and recipient SCID (severe combined immunodeficient) mice receiving naive and immune splenocytes.

**Methods:** C57BL/6 SCID mice were recipients of immune or naive splenocytes from conventional C57BL/6 mice. All mice were either inoculated with *H.pylori*, SS1 or left uninoculated. Gastric tissue was stained by immunohistochemical (CD4, CD8 and CD19) and histological techniques and scored, and serum was evaluated for anti-*H.pylori* IgG and IgM levels by ELISA at 5, 6,8 and 12 weeks post inoculation (PI).

**Results:** CD4+cell scores were elevated in inoculated conventional and recipient SCID mice (immune splenocytes), but became elevated later in inoculated recipient SCID mice (naive splenocytes). CD8+ and CD19+ cell scores remained low in all groups, and there was no significant difference between recipient SCID and conventional mice. Anti-*H.pylori* IgG was significantly elevated only in inoculated conventional mice. Anti-*H.pylori* IgM was present in all inoculated mice.

**Conclusions:** Rapid development of severe gastritis in recipient SCID mice is correlated with influx of CD4+ T-lymphocytes. CD4+ cells are involved in the inflammation. The lack of a significant difference between CD4+ cell scores in inoculated conventional mice and inoculated recipient SCID mice (immune splenocytes) while CD4+cell scores of inoculated recipient SCID mice (naive splenocytes) lagged behind indicates a rapid homing of *H.pylori* primed CD4 cells to the stomach and delayed homing of unprimed CD4 cells via secondary lymphoid tissue. Priming of CD4+ cells occurs in-situ.

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HP12

Isolation and characterization of a novel *Helicobacter* sp isolated from gastric mucosa of dolphins

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It is known that dolphins have a clinical syndrome consistent with peptic ulcer disease (PUD). Considerable speculation exists as to whether this marine mammal has *Helicobacter* sp associated gastritis and PUD. Dolphins that were 'stranded' on the east coast of the US and subsequently died were necropsied. Stomachs were submitted for microaerobic culture. Gram negative bacteria with fusiform morphology were isolated from the gastric mucosa of dolphins. Biochemically, the bacteria were urease, catalase and oxidase positive. Pure culture of the bacteria from 2 dolphins were classified by 16S rRNA analysis. It was determined that the bacteria clustered with gastric helicobacters and represent a novel *Helicobacter* sp most closely related *to 'H. heilmannii'*. To our knowledge this represents the first isolation and characterization of a novel *Helicobacter* sp from a marine mammal and emphasizes the wide host distribution and pathogenic potential of this increasingly important genus.

**HP13**

Modulation of the Th1/Th2 Response with *Heligomosomoides polygyrus* and *Helicobacter felis* coinfection in Mice: An Animal Model to Study the 'African Enigma'. JG FOX[1], P BECK[2], CA DANGLER[1], T WANG[2], MT WHARY[1], HN Shi[2], C NAGLER-ANDERSON[2]. [1]Massachusetts Institute of Technology, Cambridge, MA; [2]Massachusetts General Hospital, Boston, MA

The effect of infection with the intestinal nematode *H. polygyrus* (Th2-biased mucosal immune response) on *H. felis* associated gastritis (Th1 mediated response) in mice was studied to dissect the etiopathogenesis of the 'African enigma'. Six week old C57BL/6 female mice were divided into 4 groups consisting of control, *H. polygyrus* infected, *H. felis* infected, coinfected *with H. polygyrus* and *H. felis*. Mice infected with *H. polygyrus* were inoculated 4 weeks prior to *H. felis* infection. Gastric cytokine mRNA expression was determined in all mice 16 weeks following *H felis* (*Hf*) or *H. felis/H. polygyrus* (*Hf/Hpg*) coinfection by semi-quantitative RT-PCR using GAPDH as an internal control in all PCR reactions. The most striking finding is that mice coinfected with both *Hf* and *Hpg* have significantly ($p<0.02$-$0.05$) lower levels of TNFα, INFγ and IL-1β mRNA expression compared to mice infected with *Hf* alone. Furthermore, mice with *Hf/Hpg* coinfection had significantly ($p<0.05$-$0.01$) increased levels of IL-4, IL-10 and TGFβ compared to those infected with *Hf* alone. No statistical differences were noted in levels of expression of IL-5, IL-6, IL-12 between the *Hf* infected mice with or without *Hpg* coinfection. In summary, mice with *Hf/Hpg* coinfection had a cytokine profile suggestive of Th2 response whereas mice infected with *Hf* alone displayed a pattern of cytokine expression suggestive of a Th1 response. Such differential cytokine expression patterns may underlie the noted differences in inflammation and corpus atrophy noted in mice infected with *Hf* versus *Hf/Hpg* coinfection (see abstract # by Fox et al).

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**HP14**

Solving the African Enigma: coinfection with an intestinal helminth modulates inflammation and reduces gastric atrophy in a mouse model of Helicobacter infection. JG FOX[1], CA DANGLER[1], T WANG[2], P BECK[2], HN Shi[2], C NAGLER-ANDERSON[2] [1]MIT, Cambridge, MA; [2]MGH, Boston, MA

The effect of *H. polygyrus* (*Hpg*) on *H. felis* (*Hf*) associated gastritis in mice was studied to dissect the etiopathogenesis of the 'African enigma' which suggests that Africans have a lower rate of atrophy and gastric cancer despite very high prevalence of *H. pylori* infection. Six week old C57BL/6 female mice were divided into 4 groups consisting of control, *Hpg* infected, *Hf* infected, coinfected with *Hpg* and *Hf*. Mice infected with *Hpg* were inoculated 4 weeks prior to *Hf* infection. Pre-infection with *Hpg* resulted in an altered histopathologic response to *Hf* infection at 8 and 16 weeks post *Hf* infection. In *Hf/Hpg* infected mice mucosal hyperplasia with parietal and chief cell loss was mild at both intervals. Glandular atrophy was diminished ($p<0.05$) at 16 WPI compared to the *Hf*-only group. In contrast, the *Hf*-only group had intense hyperplastic gastritis of the corpus, characterized by gastric pit epithelial hyperplasia, mucous metaplasia and marked loss of parietal and chief cells. In the coinfected mice, 8 WPI lymphoid responses were significantly lower and granulocytic infiltration was significantly higher in comparison to *Hf*-only mice. At 16 WPI, chronic lymphoid inflammation was increased to moderate in intensity; however, the infiltrate was often intramucosal unlike the submucosal distribution observed in *Hf*-only mice. In WS stained sections at 16 WPI, *Hf* colonization of the gastric mucosa was typically dense in dual-infected mice, but was imperceptible to low in the *Hf*-only mice. *Hf/Hpg* coinfected mice have marked reduction in *Hf*-associated corpus atrophy despite chronic inflammation and high helicobacter colonization. Thus intestinal helminth infections could influence progression of *H. pylori* gastritis and provide a protective effect against development of atrophy and gastric cancer.

HP15

Novel *Helicobacter* spp isolated from colonic tissue of Macaques with chronic idiopathic colitis JG FOX[1], L HANDT[2], S XU[1], FE DEWHIRST[3], CA DANGLER[1], S MOTZEL[2], H KLEIN[2] [1]Massachusetts Institute of Technology, Cambridge, MA; [2]Merck Research Laboratories, West Point, PA; [3]Forsyth Institute, Boston, MA

Enterohepatic *Helicobacter* spp have been linked to inflammatory bowel disease (IBD) in immune dysregulated mice and have been recently isolated from cottontop tamarins with chronic colitis. Selected helicobacters are also associated with colitis and proctitis in immunocompromised humans. Chronic, idiopathic diffuse colitis is a well recognized clinical and pathological entity in captive rhesus monkeys. A group of six rhesus monkeys were diagnosed with clinically debilitating, chronic diarrhea. Histologically, colonic tissues were characterized as chronic, moderate to severe colitis and typhlitis, with diffuse mononuclear inflammation of lamina propria, reactive lymphoid hyperplasia, and focal microabscesses. Colonic tissue was cultured for *Salmonella* spp and *Shigella* spp; all results were negative. Samples were also negative for *Clostridium difficile* A and B toxins, and special stains of colonic tissue for acid fast bacteria were negative as well. In addition, the monkeys tested were negative for serum IgG antibodies to Herpes B, STLV, SRV and SIV. Microaerobic cultures grown at 37 °C and 42 °C revealed pinpoint or spreading colony growth on antibiotic impregnated media. Bacteria were identified as gram negative, oxidase positive, catalase and urease negative. Complete 16S rRNA analysis of 5 isolates indicated that the organisms isolated were novel *Helicobacter* spp. This is the first report describing novel *Helicobacter* spp being isolated from inflamed colons of rhesus monkeys. Studies dissecting the causal role of *Helicobacter* spp in initiation and progression of chronic colitis in macaques may prove useful in understanding the etiopathogenesis of IBD in humans.

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**The *Helicobacter pylori urel* protein is not involved in urea transport**

HP16

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The most characteristic enzyme of *H. pylori* is a potent multisubunit urease that is crucial for its survival at acidic pH. Six genes have been identified in the urease gene cluster, similar to other urease-producing bacterial species. The structural genes *ureA* and *ureB*, accessory genes *ureE*, *ureF*, *ureG* and *ureH* that play a role in activation of the apoenzyme and insertion of Ni2+ ions into the active site of the nascent enzyme during assembly, and *urel* whose function is not clearly understood. Previous studies have shown that the product of *urel* is not essential for the synthesis of active urease. However, non-polar mutations in *urel* result in reduced survival of *H. pylori* in acidic conditions. Based on these observations two possible roles for *urel* have tentatively been proposed 1) Transport of urea into the cytoplasm 2) Export of excess intracellular ammonia/ammonium. The aim of the present study was to assess the role of *urel* as a urea transporter. *Urel* mutant and urease-ve but *urel* positive strains of H. pylori were incubated in the presence of $^{14}C$ labeled urea for a period of 5-30 minutes at 37° C in a micoraerophillic environment. At the end of incubation the cells were harvested at 5000 X g for 10 minutes. $^{14}C$ counts of the supernatant and pellet were measured. The $^{14}C$ counts for the *urel* mutant strain and the urease-ve but *urel* positive strains were the same suggesting that *urel* mutant is able to transport urea across the membrane. Further, we incubated urease-ve but *urel* positive strain of H. pylori in the presence of $^{14}C$ urea under two different pH conditions (pH 5.5 and pH 6.5) for a period of 30 minutes at 37°C in a microaerophilic environment. At the end of incubation we measured $^{14}C$ label in the supernatant and the pellet fractions. We found no difference in the $^{14}C$ counts between the pellets of urease-ve but *urel* positive strains of *H. pylori* incubated either at pH 5.5 or at pH 6.5. These data suggest that *urel* is not a transporter of urea but may possibly be a transporter of intracellular ammonia/ammonium.

NOTES

**HP17** ***Helicobacter pylori*** **requires active hydrogenase product to survive under acidic environment**

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The peptic ulcer causing bacterium *Helicobacter pylori* grows under microaerobic (reduced oxygen concentration), using oxygen as terminal electron acceptor for respiration. It is known that *H. pylori* does not grow under anaerobic condition. But *H. pylori* genome contains all four structural genes (ORFs HP0631-0634) for functional hydrogenase which carry out the reversible reaction, $2H^+ + 2e^- \leftrightarrow H_2$ allowing them to respire under anaerobic condition. Survival of *H. pylori* under anaerobic condition was studied by incubating bacteria for varying period of time without molecular oxygen. We did not find decrease in viable count indicating that the bacteria do survive under anaerobic condition. To investigate the role of hydrogenase in bacterial respiration and survival in acidic condition, an insertion mutant of hydrogenase gene was generated. Hydrogenase deficient bacteria showed a minimal survival than the wild type at pH 2.0 in presence of 5 mM urea. Mutants are also impaired in survival in anaerobic condition, indicating requirement of hydrogenase for bacterial survival. The significantly low survival at pH 2.0 with urea, is an important clue for bacterial survival in acidic gastric environment. In conclusion, *H. pylori* requires functional hydrogenase activity to survive in anaerobic condition. Moreover, the bacteria may use $H^+$ as terminal electron acceptor, enabling survival under acidic pH in gastric environment.

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**HP18** **Molecular Disruption of Gastric Mucosal Tight Junctions by** ***Helicobacter pylori***

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*Helicobacter pylori*, the definitive gastric carcinogen of mankind, is associated with peptic ulcer disease, gastric carcinoma and lymphoma. Disruption of gastric epithelial tight junction (TJ) and mislocalization of tight junction proteins, Occludin and ZO-1, are likely to increase mucosal permeability, predisposing peptic ulceration. Gastric antral epithelial tight junctions were analyzed by transmission electron microscopy (TEM) in humans infected or uninfected with *H. pylori*. No significant ultrastructural differences in intercellular junctions or contacts were observed. Immunohistochemistry was also performed with antibodies against Occludin and ZO-1. In uninfected individuals (n=6), the distribution of Occludin and ZO-1 was linear, forming "honey comb" like pattern, indicating well defined and intact TJs. In all *H. pylori* infected individuals (n=10), distribution of Occludin and ZO-1 was diffuse and irregular, particularly within superficial gastric pits. The luminal lining epithelium was also found indistinguished in some instances. But normal localization of desmosomes within infected gastric mucosa demonstrates that *H. pylori* induce specific alterations in subcellular distribution of Occludin and ZO-1. In search of *H. pylori* factors inducing TJ disruption Vac, Cag and Urease negative mutant *H. pylori* supernatant factors were tested on T84 cell lines, exhibiting significant Occludin and ZO-1 disruption. Preliminary characterization showed >100, heat labile protein factor(s), induce disruption of nascent but not pre-existing TJs. Therefore, significant disruption of antral mucosal TJs occur at the molecular level, either by direct effect of bacterial proteins or by accompanying inflammations. This molecular injury permits antigen trafficking across gastric mucosa, allowing increased diffusion of nutrients and urea in gastric lumen, thus permits increased bacterial survival. These may lead to peptic ulceration and gastric carcinoma.

HP19

Implication of *H. pylori* OmpA in the Pathogenesis of Peptic Ulcer.

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Background. In a previous study, coincubation of AGS cells with *H. pylori* strain SS1 resulted in increased levels of gastrin promoter activity. The *H. pylori* protein responsible for the bacteria's ability to affect gastrin gene expression was identified as a previously unknown 40 kDa protein, designated OmpA for its similarity to outer membrane proteins in Gram-negative bacteria. Aim. To determine if other *H. pylori* strains contain the *ompA* gene. Methods. To isolate the *ompA* gene from other *H. pylori* strains, degenerate PCR primers were designed to amplify two regions of the gene encoding protein domains that are highly conserved among outer membrane proteins. Results. Among the strains tested were 19 clinical isolates of *H. pylori* from Australian patients. Sequence analysis of PCR fragments generated with degenerate primers revealed that one of these strains contained a gene that was highly homologous to the *ompA* gene of *H. pylori* SS1. The OmpA protein from this strain, isolated from a Greek patient with non ulcer dyspepsia, will be further characterized and compared to the OmpA protein from *H. pylori* SS1. Conclusions. The OmpA protein of *H. pylori* SS1 is present in other strains of *H. pylori* and may contribute to the pathogenesis of duodenal ulceration by its ability to stimulate IL-8 and gastrin gene expression.

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HP20

*H. pylori* OMP A Stimulates Proliferation and IL-8 Production in Gastric Cell Line.

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Background: *Helicobacter pylori* does not directly invade the mucosa but instead triggers a mucosal reaction by secreting virulence factors or by direct contact. One response to colonization by *H. pylori* is the production of IL-8 and the hormone gastrin. We have recently cloned a novel outer membrane protein from the SS1 *H. pylori* strain that is secreted and is heat stable. The protein has a C-terminal domain homologous to the outer membrane protein A from other gram negative organisms and therefore has been designated *H. pylori* OMP A. Methods: The effect of stably transfecting the full-length translated versus the secreted form of OMP A was compared to transfection of the empty pCMV vector. Both IL-8 production measured by RIA and DNA content determined by flow cytometry were analyzed. Production of the OMP A protein in transfected cells was verified by immunoblot using antibody generated against conserved peptide domains. Results: The results show that there was an increase in IL-8 production with the full-length (3.5-fold) and secreted (2.5-fold) OMP A forms compared to empty vector or untransfected cells. Similarly, both forms of OMP A stimulated an increase in S phase (DNA content) and decrease in G1 (increased cell progression) compared to cells transfected with the empty vector or nontransfected cells. Conclusion: *H. pylori* OMP A is a novel secreted outer membrane protein that stimulates mammalian cell IL-8 production and cell proliferation.

HP21 **Analysis of the Urease Gene Cluster of *Helicobacter hepaticus***

C.S. BECKWITH, L.K. RILEY, University of Missouri, Columbia, MO

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The mouse pathogen *Helicobacter hepaticus* was first identified in 1992 when it was found to cause disease in laboratory mice. *H. hepaticus* is a gram negative, microaerophilic, urease positive spiral rod with sheathed flagella. *H. hepaticus* colonizes the cecum, colon, and liver, and unlike the human pathogen *H. pylori*, it does not colonize the stomach. Urease has been identified as an important virulence factor in *H. pylori*. In order to study the role of urease in the pathogenesis of disease caused by *H. hepaticus*, we cloned, sequenced, and expressed genes of the *H. hepaticus* urease cluster. Clones containing *H. hepaticus* urease structural genes were identified by hybridization of a *H. hepaticus* gene library with a probe amplified from the *H. pylori ureA-B* genes. Urease accessory genes were amplified by PCR. Primers were designed to amplify the entire *H. hepaticus* urease cluster which was then cloned into a single plasmid. Clones were sonicated and soluble proteins were separated by SDS-PAGE and subjected to Western blot analysis. Immunoreactive bands at approximately 27 kDa and 60 kDA were identified with antibodies to *H. pylori* ureA and ureB, respectively, which confirmed that the correct gene cluster had been cloned and expressed. Analysis of the sequenced genes determined that the *H. hepaticus* urease cluster contains homologs of *H. pylori* structural genes *ureA* and *ureB*, and accessory genes *ureI*, *ureE*, *ureF*, *ureG*, and *ureH*. The order of these genes is conserved in *H. hepaticus*, and the deduced amino acid sequences have significant homology to urease genes of other members of the *Helicobacter* genus. Despite its different host range and tissue tropism, *H. hepaticus* produces a urease closely related to the urease of *H. pylori*.

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HP22 Protective Role Of Cyclooxygenase-2 In *H. pylori* Gastritis: Exacerbation of Inflammation in COX-2 Knockout Mice. X-L LUO, KS RAMANUJAM, H-M ZHAO, SF MOSS, RG RUSSELL, CB DRACHENBERG, AND KT WILSON. University of Maryland School of Medicine, Baltimore, MD, USA

We have demonstrated that *H. pylori* (*Hp*) stimulates expression of cyclooxygenase (COX)-2, both in human stomach and in *in vitro* models. Since COX-2 activity can inhibit Th1 cellular immune responses and epithelial apoptosis, we hypothesized that COX-2 downregulates the severity of *Hp* gastritis. **Methods:** C57BL/6,Sv129 COX-2 -/- and COX-2 +/+ mice were inoculated with mouse-adapted strains of *Hp* (SS1 or G1.1) and gastric tissues obtained at 2 and 4 mo (n = 5-7 per group). We assessed: histologic gastritis, graded by the Sydney system; colonization density; gastric COX-2 expression; apoptosis, by TUNEL and Bak staining; and tissue cytokine levels, by RT-PCR and ELISA. **Results:** *Hp* infection increased COX-2 mRNA and protein expression and $PGE_2$ levels (4-fold) in the +/+ mice; this did not occur in the -/- mice. COX-2 -/- mice had significantly increased acute and chronic inflammation in both antrum and body (see table), duodenal ulcers, and 2 log order more *Hp*/mg tissue protein. In the COX-2 -/- mice, TUNEL and Bak staining, as well as IL-12, IFN-γ, and IL-1 tissue cytokine levels were increased 2- to 4-fold.

Histologic grading of gastritis (0-3 scale, *Hp* strain SS1, gastric antrum)

| | 2 mo | | 4 mo | |
|---|---|---|---|---|
| | Acute | Chronic | Acute | Chronic |
| COX-2 +/+ | 1.3 ± 0.2 | 0.7 ± 0.2 | 1.3 ± 0.1 | 0.5 ± 0.2 |
| COX-2 -/- | 2.4 ± 0.2** | 1.5 ± 0.2* | 2.9 ± 0.1*** | 1.8 ± 0.3** |

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. COX-2 +/+ mice.

**Conclusions:** COX-2 deletion exacerbates the effect of *Hp* infection, indicating that COX-2 inhibits *Hp* gastritis. Protective effects of COX-2 include downregulation of *Hp* colonization, apoptosis, and Th1-mediated inflammatory responses.

## The Role of the Lipopolysaccharide in Adhesion of *Helicobacter pylori* To Gastric Mucosa

HP23

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The role of lipopolysaccharide in the interaction of *H. pylori* NCTC11637 with gastric mucosa was investigated. This was achieved by insertional mutagenesis of *galE* and *rfbM* which are prerequisites for the biosynthesis of UDP-galactose and GDP-fucose, respectively. By SDS-PAGE of purified LPS samples and immunoblotting with anti Lewis X Mabs it was demonstrated that the *galE* mutant lacked O-antigen and expressed a rough LPS. In contrast the *rfbM* mutant expressed O-antigen, but no longer reacted with anti-Lewis X Mabs, which is consistent with the absence of fucose. The precise chemical structure of the LPS synthesised by each mutant is presented. Both mutants were impaired in ability to adhere to gastric biopsy samples obtained from the antrum region of an anatomically normal human male. This suggested that LPS and more specifically the O-antigen played an important role in this interaction. This hypothesis was confirmed by demonstrating that synthetic Lewis X, conjugated to latex beads, bound to gastric mucosa in a manner identical to wild type *H. pylori*.

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## C-terminal processing of *Helicobacter pylori* VacA analyzed by MALDI TOF mass spectrometry

HP24

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The only known toxin secreted by *H. pylori* is designated VacA (vacuolating toxin). The *vacA* gene in *H. pylori* strain 60190 encodes a predicted precursor protein of 139 kDa, consisting of 1287 amino acids. This protoxin undergoes cleavage of a 33-amino-acid N-terminal signal sequence and C-terminal processing to yield a mature ~90 kDa secreted toxin. VacA secretion and processing probably occur via mechanisms similar to those used for secretion of *N. gonorrhoeae* IgA protease, but the site of C-terminal VacA cleavage and the relevant protease mediating this cleavage are not known. To investigate these questions, mass spectrometric analysis of purified VacA was performed using a Voyager Elite MALDI TOF mass spectrometer (PerSeptive Biosystems) in the linear delayed-extraction mode. Mass spectrometry measurement of undigested VacA yielded a peak of average mass 87,836 daltons. The 87,836 dalton form of VacA spontaneously degraded into fragments with molecular masses of 54,356 and 33,321 daltons. To precisely characterize the C-terminus of VacA, the toxin was digested with cyanogen bromide, and the fragments were analyzed by mass spectrometry. Seven different peptides of similar size were detected, all of which can possibly be derived only from the C-terminus of VacA. The sizes of these peptides allow us to deduce that cleavage occurs after residues 846, 847, 848, 849, 850, 851, and 854 of the protoxin. We hypothesize that the primary proteolytic cleavage event occurs between amino acids 854 and 855, and that several individual amino acids are thereafter susceptible to further cleavage. Mass spectrometric analysis of the size of mature VacA provides a necessary first step in understanding the structure and processing of this important bacterial toxin.

**HP25** *Helicobacter pylori luxS* is required for the production of an extracellular quorum sensing signal

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NOTES

Regulation of gene expression in response to bacterial cell density (quorum sensing) occurs in many bacterial genera. In this study, we used the luminescent *Vibrio harveyi* strain BB170 (kindly provided by B. Bassler) as an indicator in order to determine whether *H. pylori* produces extracellular signaling molecules. Cell-free culture supernatants from both *H. pylori* strain 60190 and strain 26695 induced 78- and 244-fold higher luminescence, respectively, in *V. harveyi* than did sterile culture medium. Maximal production of the signaling molecule by *H. pylori* occurred in the mid-exponential phase of growth, with a loss of activity throughout stationary phase. The *H. pylori* signaling molecule had a molecular mass of <10 kDa, and its activity was lost upon heating to 100°C but only partially lost at 80°C. The genome sequence of *H. pylori* 26695 does not contain any genes predicted to encode acyl-homoserine lactone synthetases, but contains a homologue of *luxS*, which is required for production of autoinducer-2 (AI-2) signaling molecules in *V. harveyi*, *E. coli*, and *S. typhimurium*. To evaluate the role of *luxS* in *H. pylori*, we constructed *luxS* null mutants in both *H. pylori* 60190 and 26695. Conditioned media from the wild-type *H. pylori* strains induced >100-fold greater luminescence in the *V. harveyi* assay system than did conditioned medium from either mutant strain. These data indicate that *H. pylori* produces an extracellular signaling molecule with features similar to the recently described AI-2 molecule. We speculate that AI-2 regulates gene expression in *H. pylori* in response to changes in bacterial cell density.

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**HP26** The role of *ureI* in acid tolerance of *Helicobacter pylori*

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*H. pylori,* a neutralophile, is able to tolerate gastric acidity by regulation of cytoplasmic, not surface, urease activity. Cytoplasmic urease is inactive at neutral pH but activates at pH <6.5 and remains active down to pH 2.5, whereas surface urease is inactive at pH < 4.0. Urea addition to organisms at acidic pH results in a pH increase of the periplasmic space (6.2) and an elevation of the transmembrane potential (-101 mV) (Scott et al,1998). The *Hp* urease gene cluster is composed of 7 genes: ureA and B encode for the structural subunits of urease and E, F, G and H are involved in urease assembly (Ferrero and Labigne, 1993). The 7$^{th}$, *ureI,* is an integral 6 segment membrane protein as shown by *in vitro* translation, with two large periplasmic loops between TM2/3 and 4/5. Non-gastric *Helicobacters* do not express *ure*I, nor show acid activation of cytoplasmic urease or increase of membrane potential with urea. Deletion of *ureI* results in a loss of acid activation of cytoplasmic urease and of the increase in the membrane potential with urea addition. 0.01% $C_{12}E_8$ in intact organisms activates urease at neutral pH. The urea dependent elevation of periplasmic pH as visualized in wild type organisms by confocal microscopy of the non-permeant pH dye, BCECF, at pH 5.5, is abolished in *ureI* knockout mutants. Cytoplasmic fluorescence appears in these mutants following addition of $C_{12}E_8$ at pH 5.5. *ureI* appears to encode a urea transporter. cRNA derived from the *ureI* gene injected into *Xenopus* oocytes, results in a non-saturable, specific, uptake of urea, but not thiourea, only under acidic conditions. UreI protein is a channel-like urea transporter present in the inner membrane of *H. pylori*, activated by acid on its periplasmic surface. Regulation of cytoplasmic urease activity by the presence of this acid activated urea transporter is essential for gastric survival and colonization by *Hp* (Skouloubris et al,1999). Inactivity of ureI at pH > 6.5 allows survival of intact *H. pylori* at neutral pH in the presence of urea.

HP27

*helA* and *helB*, Two Putative Subunits of a DNA Helicase in the *cag* Pathogenicity Island, Indirectly Modulate *Helicobacter pylori* Urease Activity

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*H. pylori* urease is a nickel-requiring metalloenzyme that hydrolyzes urea to $NH_3$ and $CO_2$. *hp0548*, a gene at the right junction in the *cag* pathogenicity island (PAI), encodes a predicted DNA helicase that significantly enhanced urease activity ($p<0.05$) in *E. coli* containing *H. pylori* urease and the *nixA* nickel transporter provided on plasmid pHP8080. *helA* and *helB* are two predicted open reading frames of *hp0548* in strain 43504, in contrast with four other strains of *H. pylori*, including 26695. These latter strains all have a predicted frame shift in *hp0548*, which potentially renders *hp0548* nonfunctional. Separate subclones of either *helA* or *helB* conferred urease-enhancing activity to *E. coli* (pHP8080). When *helA* was disrupted, urease-enhancing activity was lost in *E. coli* (pHP8080). Surprisingly, a confirmed *H. pylori* strain 26695 isogenic mutant of *helB* had enhanced (rather than reduced) urease activity compared with the wild type strain. Indirect modulation of urease activity was supported by the lack of differences in β-galactosidase activity by any *hel* subclone in *E. coli* containing an *H. pylori ureA* promoter-*lacZ* fusion on a low copy plasmid. Our results suggest that *helA* and *helB* from the *cag* pathogenicity island are indirect urease-modulating factors of *H. pylori*. Thus, *H. pylori* urease expression may not be constitutive. We are currently purifying the HelA and HelB proteins to investigate their precise role in modulation of urease activity.

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HP28

Pleiotropic phenotypes of *Helicobacter pylori* arginase mutants: changes in amino acid catabolism activities and increased sensitivity to acid.

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*H. pylori* arginase hydrolyzes L-arginine to L-ornithine and urea. Urease hydrolyzes urea to bicarbonate and ammonium, which neutralize acid. Both enzymes are involved in *H. pylori* nitrogen metabolism. The roles of arginase in the physiology of *H. pylori* were investigated *in vitro* and *in vivo* since arginase in *H. pylori* is metabolically upstream of urease and urease is known to be required for colonization of animal models by the bacterium. The *H. pylori* gene *hp1399*, orthologous to the *Bacillus subtilis rocF* gene encoding arginase, was cloned and isogenic allelic exchange mutants of three *H. pylori* strains were made using two different constructs: 236-2 and *rocF::aphA3*. In contrast to wild type (WT) strains, all *rocF* mutants were devoid of arginase activity and had diminished serine dehydratase activity, an enzyme activity which also generates ammonium. Compared with WT strain 26695 of *H. pylori*, the *rocF::aphA3* mutant was ~1000-fold more sensitive to acid exposure. The acid sensitivity of the *rocF::aphA3* mutant was not reversed by addition of L-arginine, in contrast with the WT (~10,000-fold difference in viability). Urease activity was similar in both strains and both survived acid exposure equally well when exogenous urea was added, indicating that *rocF* is not required for urease activity *in vitro*. Finally, *H. pylori* mouse-adapted strain SS1 and the 236-2 *rocF* isogenic mutant colonized mice equally well, 8/9 mice vs 9/11, respectively. However, the *rocF::aphA3* mutant of strain SS1 had moderately reduced colonization (4/10 mice). The geometric mean of *H. pylori* recovered from these mice (in $\log_{10}$ CFU) was 6.1, 5.5 and 4.1, for the WT and two mutants, respectively. Thus, *H. pylori rocF* is required for arginase activity and is crucial for acid protection *in vitro*, but is not essential for *in vivo* colonization of mice or for urease activity.

**HP29** The *Helicobacter pylori flbA* Flagellar Biosynthesis/regulatory Gene is a Urease-decreasing Factor of *H. pylori* and *Proteus mirabilis* Urease Expressed in *E. coli*. **C. COKER***, D. J. MCGEE, J. M. HIMPSL, R. S. EL-ASADY, AND H. L. T. MOBLEY. Univ. of MD Sch. of Med., Dept. Microbiol. & Immunol., Baltimore, MD

**NOTES**

*H. pylori* and *P. mirabilis* ureases are nickel-requiring metalloenzymes that hydrolyze urea to $NH_3$ and $CO_2$. The *H. pylori flbA* gene, a flagellar biosynthesis/regulatory gene homologous with *Yersinia spp. lcrD*, decreased urease activity 15-fold ($p<0.0001$) in *E. coli* containing the *H. pylori* urease gene cluster and the NixA nickel transporter provided on plasmid pHP8080. Urease activity was restored to original levels when the *flbA* gene was disrupted with a kanamycin cassette (*aphA3*). The effect of *flbA* on the *P. mirabilis* urease was investigated by introducing a low-copy-number *flbA* recombinant plasmid (p*flbA*) into *E. coli* containing a plasmid encoding the *P. mirabilis* urease transcriptional activator *ureR* and the *ureD* promoter fused to *lacZ*, which encodes β-galactosidase. We observed a 4-fold decrease in β-galactosidase activity produced by this *E. coli* strain containing p*flbA* versus the plasmid vector control strain ($p<0.001$). When p*flbA* was provided *in trans* in either *E. coli* containing the *P. mirabilis* urease gene cluster or in wild-type *P. mirabilis* HI4320, decreased urease activities vs control strains of 3-fold ($p<0.001$) and 1.5-fold ($p<0.001$) respectively, were observed. We have purified the C-terminal putative cytosolic domain of FlbA ($FlbA_{cyt}$) to determine whether other *H. pylori* proteins interact with $FlbA_{cyt}$ or are involved in modulation of urease activity. Preliminary experiments suggest that we have tentatively obtained an isogenic *flbA* mutant of *H. pylori* that has altered urease activity. Since FlbA/FlhA regulate flagellar biosynthesis and secretion and form a structural component of the flagellar secretion apparatus, our results suggest that motility and urease activity are coupled in both *H. pylori* and *P. mirabilis*.

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**HP30** **Cloning and Characterization of an α 1-3/4 fucosyltransferase of *Helicobacter pylori***

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The gastric pathogen *Helicobacter pylori* can express the histo blood group antigens which are on the surface of many human cells. Most *H. pylori* strains express the type II carbohydrates, Lewis X and Y, while a small population express the type I carbohydrates, Lewis A and B. The expression of Lewis A and Lewis X, as in the case of *H. pylori* strain UA948 requires the addition of fucose in α1-4 and α1-3 linkages to type I or type II carbohydrate backbones respectively. This work describes the cloning and characterization of a single *H. pylori* fucosyltransferase (*fucT*) enzyme which has the ability to transfer fucose to both of the aforementioned linkages in a manner similar to the human fucosyltransferase V (Fuc-TV). Two homologous copies of the *fucT* gene have been identified in each of the genomes sequenced. Characteristic adenosine and cytosine tracts in the amino terminus and repeated regions in the carboxy terminus are present in the DNA of the two UA948*fucT* genes, but the genes also contain differences when compared to previously identified *H. pylori fucTs*. The UA948*fucTa* gene encodes an approximately 52 kDa protein containing 475 amino acids, while UA948*fucTb* does not encode a full length *FucT* protein. *In vitro*, the enzyme encoded by UA948*FucTa* appears to add fucose with a greater than 5 fold preference for type 2 acceptors, but still retains significant activity using type 1 acceptors. The addition of the fucose to the carbohydrate backbone, by the UA948*FucTa* enzyme, does not appear to be affected by fucosylation at other sites on the carbohydrate chain. Through mutational analysis we demonstrate that only a single *fucT* is active in this *H. pylori* isolate and inactivation of this enzyme eliminates expression of all Lewis antigens.

## *In Vivo* Lewis Antigen Switching by *Helicobacter pylori* HP31

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Background and Aims: The expression of Lewis antigens by the gastric pathogen *Helicobacter pylori* in serial biopsy isolates was investigated to assess antigen expression and stability. Subjects were part of a safety and efficacy trial for the anti-adhesive carbohydrate therapy, 3`sialyllactose (3`SL).

Material and Methods: 26 Asymptomatic subjects were administered various doses of 3`SL for up to 56 days and gastric biopsies were performed on a regular basis during the dosing period, as well as 30 days post dosing. *H. pylori* strains were isolated from the biopsy samples and the expression of Lewis antigens by *H. pylori* was investigated by ELISA and immunoblot. The genomic profiles of the *H. pylori* isolates obtained were investigated by random primer-PCR.

Results: The 26 subjects provided 127 *H. pylori* culture-positive biopsy samples and the Lewis antigen expression was assessed. A high proportion of subjects (14/26) yielded sequential *H. pylori* isolates which appeared genetically identical by random primer-PCR but had variable Lewis antigen expression. In contrast, one subject was colonized by two genetically distinct isolates also exhibiting variable Lewis antigens. Isolates from the remaining 11 subjects exhibited stable Lewis antigenic phenotypes. The proportion of subjects with *H. pylori* isolates not expressing Lewis antigens identified is higher than previously reported (10/26).

Conclusions: The *in vivo* expression of the Lewis antigens by *H. pylori* appears to less stable *in vivo* than that previously reported *in vitro* studies. We demonstrate that infection by a single strain of *H. pylori* which can alter its Lewis antigen expression occurs at a higher rate than previously estimated. Subjects colonized by *H. pylori*, that do not express Lewis antigens demonstrate that Lewis antigens are not a requirement for the colonization of humans.

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## Functional genomics of *Helicobacter pylori*: generation of mutants with altered lipopolysaccharide. HP32

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Using the complete annotated genome sequence of *H. pylori* 26695 we identified potential glycosyltransferases using BLAST analysis and then mutated genes likely involved in the biosynthesis of the O chain region of LPS. Isogenic mutants of *H. pylori* generated from a number of strains were examined and compared to their respective parents by SDS-PAGE and immunoblotting to determine the effect on LPS electrophoretic mobility. Structural analysis of LPS isolated from the mutants confirmed that O chain synthesis had been affected and revealed the precise structure of the truncated molecule. Functional characterisation of the PCR cloned gene product was also performed. NMR analysis confirmed the expected linkage in the product obtained from preparative synthesis. The mutation was introduced into *H. pylori* strain SS1 and mouse colonisation experiments were conducted to investigate the requirement for O chain for gastric colonisation. The mutant strain colonized to a much lower level than the wild-type strain. The ability to produce structurally defined, truncated LPS molecules should facilitate investigations of the biological role of LPS in pathogenesis and more clearly define its potential as a vaccine or therapeutic target.

**HP33** Dissecting the *Helicobacter pylori* Vacuolating Cytotoxin Using Sliding Complementation Analysis within Target Cells

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*Helicobacter pylori* secrete multiple protein factors contributing to colonization, survival, and damage to the host. The only exotoxin identified, however, is the vacuolating cytotoxin (VacA) that induces the formation of large vacuoles originating from late endocytic vesicles in sensitive mammalian cells. While evidence is accumulating that VacA is an A-B toxin, neither an intracellular target, nor a distinct A fragment has been identified. To localize the putative catalytic A-fragment, we transfected HeLa cells with plasmids encoding truncated forms of VacA fused to green-fluorescence protein. By analyzing truncated VacA fragments for intracellular vacuolating activity, we localized the minimal functional vacuolating domain to the amino terminal 422 residues of VacA, which is less than one-half of the full-length protein. VacA is frequently isolated as a proteolytically-nicked protein of two fragments that remain noncovalently associated and retain vacuolating activity. Neither the amino-terminal fragment (Fragment I; 311 residues), nor the carboxyl-terminal fragment (Fragment II; 642 residues) of proteolytically nicked VacA are able to induce cellular vacuolation by themselves. However, co-transfection of HeLa cells with plasmids encoding Fragments I and II revealed that these inactive fragments functionally complement each other within the target cell to mediate vacuolation *in trans*. We have exploited this discovery by analyzing a series of genetically split genes using sliding complementation analysis. Using this approach we have identified essential toxin determinants in both Fragments I and II. Collectively, our results suggest a novel molecular architecture for VacA, with cytosolic localization of both fragments of nicked toxin required for intracellular vacuolating activity.

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**HP34** A New Grading System for Reproducible Inter-laboratory Histologic Quantification of Gastritis in Mice Infected with *Helicobacter pylori*

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**Background and objective**: Comparison of animal models between laboratories is hindered by varying histologic scoring systems and by subjective interpretation of lesion severity. The purpose of this study was to determine if the extent of gastric lesions can be used as a reproducible method to quantify gastritis.

**Methods**: Four different investigators scored 194 histologic slides from control and *H. pylori*-infected C57BL/6 and severe, combined, immunodeficient (SCID) mouse stomachs. Severity of gastritis ranged from none to severe, depending on the mouse group. Investigators were blinded to the source of the slides and scored each microscopic field for the presence of absence of neutrophils, adenitis, gastritis, and epithelial metaplasia according to a written definition. Extent was expressed as the percent of fields containing the lesion. Investigators were considered in agreement if they scored sections within 20% .

**Results**: With few exceptions, slides with no lesions were scored negative by all investigators. Agreement as to extent of lesions was somewhat less. It was best for evaluation of gastritis (≥80% agreement), acceptable for neutrophils (70%) and least for adentitis and metaplasia (≤20%). Extent of gastritis correlated with expected severity based on mouse group.

**Conclusions**: Evaluation of extent of gastritis and neutrophils is reproducible among investigators scoring slides according to a written description. Scoring based on percent affected mucosa offers a method for inter-laboratory comparison of lesion severity .

HP35

The role of *Helicobacter pylori vacA* and *cag* genes in colonization and gastritis in germ-free mice and piglets

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**Objective**: To determine if *vacA* or *cag*-region genes promote either colonization or gastritis in germ-free mice and germ-free piglets.

**Methods**: To interrupt the *virB11*, *virB10,* and/or *vacA* genes insertion mutations were created in *H. pylori* strains 26695 (pig-passed) and SS1 (mouse-passed). To delete the entire *cag* region, strain 26695 was transformed with a DNA construct containing *cag* flanking sequences surrounding a CAT cassette. Gastritis and colonization potential of bacterial mutants were evaluated in germ-free piglets inoculated with 26695 or 26695Δ*cag* and in mice given SS1, SS1*virB11::CAT*, or SS1*virB10::CAT*. In vivo competition was determined in piglets and mice co-inoculated with both *virB11*- and *vacA*-deficient mutants.

**Results**: All *mono*-inoculated animals became colonized, and there were no differences in colonization density between groups. All *co*-inoculated animals became colonized with both strains, and there was no difference in colonization density between the strains. Three weeks after inoculation, the severity of neutrophilic infiltrate in mice colonized with SS1*virB11::CAT* was significantly greater than in mice colonized with wild-type strain SS1. Otherwise, neither *cag*-region deletion nor insertional mutation of *virB10* or *virB11* had any effect on gastritis or neutrophilic infiltrate in piglets or mice.

**Conclusions**: Neither *vacA* nor *cag*-regions genes are necessary for colonization or gastritis in piglets or mice. Inactivation of *vacA* or the *virB11* homologue does not confer a selective disadvantage during co-colonization by *H. pylori*.

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## IRON LEVELS DURING GROWTH INFLUENCE THE EXPRESSION OF *H. PYLORI* VIRULENCE FACTORS

HP36

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Outer membrane vesicles (OMV) are readily harvested from the supernatant following growth of *Helicobacter pylori* in Brucella broth supplemented with 5% fetal calf serum (BB, 5% FCS). Furthermore, our studies show that OMV of *H. pylori* type 1 strains contain biologically active vacuolating cytotoxin.

OMV release increases dramatically at the expense of bacterial growth when *H. pylori* are grown for 72 hours in BB, 5% FCS under iron-limiting conditions (50 uM Desferal). However, these OMV contain not only reduced levels of VacA but also independent expression of two proteolytic enzymes. When iron salts (50 uM) are added to counteract the effect of the Desferal, VacA levels are restored in the outer membrane whereas proteolytic activity disappears.

In conclusion, we find that OMV release by *H. pylori* is a response to environmental iron levels. Furthermore, qualitative changes that occur in outer membrane composition under iron-limiting conditions suggest that the iron status of a host may be another factor which contributes to the differing patterns of *H. pylori*-associated disease.

HP37 Analysis of flagellar biosynthesis in *Helicobacter pylori*.

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*Helicobacter pylori*, a motile bacterium isolated primarily from the human stomach, is associated with gastritis, gastric and duodenal ulcer and gastric carcinoma. Experiments in animals using isogenic mutants have shown that both the FlaA and FlaB flagellins are required for full motility and for establishing persistent infection. The *flaA* and *flaB* genes are unlinked on the chromosome and are preceded by different promoters suggesting that they may be differentially regulated. Recently FlgR, a transcriptional activator, has been shown to control, via $\sigma^{54}$-dependent promoters, five operons containing genes for flagellar biosynthesis. Scrutiny of the *H. pylori* 26695 genome sequence suggested there are over 40 genes with a role in flagellar biosynthesis. Isogenic mutants were constructed in seven of these genes and all were non-motile. Further examination by electron microscopy showed that all seven mutants were aflagellate. Northern slot hybridisation to detect flagellin mRNA and immunoblotting to detect flagellin proteins have shown differences in transcription, translation and secretion of FlaA and FlaB between both individual mutants and when compared to the wild-type strain. Studies using the *H. pylori* mouse model will test the ability of each of these mutants to colonise.

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HP38 An investigation into colonisation of the stomach by *Helicobacter pylori* using differential gene expression.

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A unique characteristic of *H. pylori* is its ability to persist in the harsh environment of the human stomach where it is exposed to conditions such as low pH and acidified nitrite. To identify genetic determinants which contribute to survival and colonisation, we studied differential gene expression in *H. pylori* by hybridisation of cDNA probes to a high density genomic DNA array. For low pH experiments, log phase *H. pylori* was incubated for 30 min. in either citrate buffer (pH 4) or PBS (pH 7). For acidified nitrite, log phase bacteria were exposed for 10 min. to either KCl/HCl buffer (pH 2) or PBS (pH 7), each containing 5 mM urea and 200 μM sodium nitrite. Radiolabelled cDNA was generated from total RNA by reverse transcription and hybridised to a *H. pylori* NCTC 11637 genomic DNA array. 58 genes were found to be differentially expressed at pH 4 compared to pH 7, and 59 genes were differentially expressed in acidified nitrite compared to nitrite at neutral pH. Among these differentially expressed genes were (i) genes that may play a role in virulence, (ii) genes encoding regulators of gene expression, and (iii) *H. pylori*-specific genes.

## Contamination of Mouse-passaged Streptomycin Resistant *Helicobacter pylori* with *Helicobacter bilis*. HP39

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**Aim.** The colonization efficiency of a mouse-passaged *Helicobacter pylori* strain SS1 was determined in mice. SS1 was made streptomycin resistant (200µg/ml, SS1-Strep) to facilitate re-isolation. Groups of 10 mice were given one, two or three doses of *c.* $2*10^8$ CFU each.

**Results.** Forty days after infection mice were killed. The average number of CFU/g stomach tissue varied from $5.9*10^7$, $9.6*10^7$ to $11.7*10^7$ in the group given three doses. A high level of specific antibodies was found in all groups. Examination of the passaged strain by electron microscopy revealed the presence of curved bacteria with unipolar flagella, presumably *H. pylori*, as well as straight rod-like bacteria with periplasmic fibrils, and bipolar flagella. The latter form consisted of *c.* 10% of bacteria in the preparation.

**Discussion.** SS1-Strep was isolated in high numbers from infected mice even if only one dose was given, however levels were significantly lower ($P$=0.03) with one dose. The level of specific antibodies in serum was dose independent. Despite the presence of streptomycin, *Helicobacter* spp. residing in the murine gastrointestinal system contaminated the mouse-passaged sample. Morphological characteristics were consistent with *H. bilis*. If passaged strains are used for inoculation in challenge experiments, results should be interpreted with care, because the presence of other *Helicobacter* spp. may alter the outcome of challenge.

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## The *Helicobacter pylori* UreI Protein Is Essential for Adaptation to Acidity and for Murine Stomach Colonization HP40

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To survive in an environment as hostile as the human stomach, *Helicobacter pylori* (*H.p*) has to be able to adapt quickly and efficiently to pH variations going from extreme acidity to nearly neutrality. This process is dependent on urease which has been shown to be activated when external pH is below 4 (Scott *et al.* 1998 Gastro. 114:58-70). We here present data showing that the UreI protein, expressed from the urease gene cluster, is required for the urease-dependent response to acidity and thus for *H.p* adaptation and resistance to low pH. To investigate the role of UreI, an *H.p* strain carrying a deletion of *urel* replaced by a non-polar $Km^R$ cassette was constructed (N6-834). We established (Skouloubris *et al.* Infect. Immun. 1998, 66: 4517-4521) that UreI is not necessary for synthesis of a functional urease but that UreI is essential for colonization of the mouse stomach by the *H.p* SS1 strain. The role of UreI in *H.p* adaptation to extracellular pH was further investigated. Survival and pH variations were measured after *in vitro* incubation in phosphate buffer at different pH values, in the presence or the absence of 10mM urea, of three *H.p* strains: the parental strain (N6), the UreI-deficient strain (N6-834), and the N6-834 mutant complemented with a pHel2 vector derivative carrying the *urel* gene. UreI was shown to be required for *H.p* resistance to acidity in the presence of urea. Kinetics of ammonium production by the three strains, incubated in the same conditions, indicated that: (i) the presence of UreI is responsible for enhanced ammonium production at low pH, (ii) this rapid UreI-mediated response to acidity is strictly specific for urease. Hypothesis on the function of UreI as an urease activator, an urea transporter or an active ammonia export system are presently tested.

HP41

## Identification of *Helicobacter pylori* virulence genes using Random Mutagenesis and Loop Amplification (RMLA)

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In this study, we describe the development of the random mutagenesis and loop amplification (RMLA) system, which has potential for identifying new gene loci involved in virulence-associated traits of *Helicobacter pylori* and for screening for factors important in establishing and maintaining gastric colonization. RMLA is based on the mini-Tn*3*-Km transposon shuttle-mutagenesis system, that is able to generate a random bank of insertion mutants of *H. pylori*. Integral to the system is a simple method for directly identifying the site of transposon insertion within the *H. pylori* chromosome, so allowing the function of unknown genes to be readily characterized. RMLA is also able to distinguish individual mutants within a pool, making it a potentially useful technique for screening comparatively large numbers of mutant strains for those with attenuated phenotype in an animal model. In a preliminary screen using a mouse model of infection, we have identified a number of chromosomal loci that may be important for colonization by *H. pylori*.

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HP42

## Preliminary Serological and Chemical Characterization of Lipopolysaccharides from non *Helicobacter pylori* helicobacters

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Although lipopolysaccharide(LPS) plays an important role in *Helicobacter pylori* pathogenesis, little information is available on the LPS structure of other *Helicobacetr spp.* The present study was undertaken to fill that void. The LPSs of *H. helmanii, H. felis, H. hepaticus* and *H. mustelae* were investigated. ProteinaseK treatment of whole cell extracts yielded LPS mini-preparations whereas larger quantities of LPS were extracted by hot phenol-water extraction and purified: Subsequently electrophoretic analysis was carried out using SDS-PAGE with silver staining. Serodot analysis using anti blood group monoclonal antibodies and polyclonal antisera versus *H. pylori* core structures were used to characterize the bacteria serologically. Chemical analysis was performed using sugar and methylation analyses of free carbohydrates. Electrophoretic analysis showed that *H. hepaticus* and *H. mustelae* both produced low molecular weight LPS. *H. felis* LPS expressed high molecular weight LPS as did *H. helmanii*. None of the LPSs tested were found to bind to anti-Lewis antibodies including *H. helmanii*, which like *H. pylori* colonizes humans. The *H. pylori* anti-core antibodies reacted with all the *Helicobacter* spp tested indicating conservancy of certain core structures within the genus. Chemical analysis was performed on LPSs from *H.felis* and *H. mustelae* which confirmed these findings. This study indicates that *H. mustelae, H. felis* and *H. hepaticus* express LPS which differs from *H. pylori* in its macromolecular properties. The core region of the *Helicobacter* spp. tested share similar epitopes with *H. pylori*. However, the O side chain of their LPSs do not present Lewis antigens serologically and this was confirmed chemically for both *H. felis* and *H .mustelae.*

## A Relationship Between the Expression of High Molecular Weight-Lipopolysaccharide by *Helicobacter pylori* and Colonizing Ability in Mice. HP43

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Subculturing of *Helicobacter pylori* on solid media may cause loss of expression of O side chains of high molecular weight lipopolysaccharide (LPS) and therefore loss of mimicry of Lewis (Le) antigens. Analysis of a non colonising *H. pylori* strain showed it produced only low molecular weight LPS and therefore we investigated the hypothesis that loss of O chain production may influence colonization in mice. Four fresh *H. pylori* clinical isolates, two lab passaged strains and the reference colonizing strain SS1 were used in this study. NMRI mice were used as the primary model and in addition C57BL/6, CBA/Ca, and BALB/cA mice were also used. Colonization was determined through culture techniques at 2 weeks post infection. LPS mini-extracts (proteinaseK-treated lysates) from *H. pylori* were analyzed by SDS-PAGE with silver-staining and immunoblotting using anti-Le monoclonal antibodies. Strains were also analyzed using a previously described lectin typing scheme. The four clinical isolates and SS1 were found to colonize mice readily, whereas CCUG 17874 and G50 did not. The colonizing strains possessed high molecular weight LPS expressing $Le^x$ and/or $Le^y$ antigens, whereas the non colonizing strains did not. However, culture of *H. pylori* CCUG 17874 and G50 in liquid media led to production of high molecular weight LPS, Le antigen expression and colonisation. Using lectin typing, non colonizing strains were untypeable whereas colonizing strains has a stable lectin type before and after colonization. Thus a relationship exists between the expression of high molecular weight LPS and the colonizing ability of *H. pylori* in NMRI mice primarily but also in other mouse models. We conclude therefore that expression of an O-side chain with Le antigen mimicry is necessary for the successful colonization by *H. pylori*.

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## Novel Structures of Lipopolysaccharides of Danish *Helicobacter pylori* Strains HP44

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Chemical and serological studies have shown that *Helicobacter pylori* strains express $Lewis^x$ ($Le^x$) and $Lewis^y$ ($Le^y$) human blood group antigens on the O-side chains of lipopolysaccharides (LPS) and that >80% of strains express these antigens. However, no information is currently available on the LPS structures of non typeable strains. The aim of the present study was to examine the structure of the LPS of strains non typeable with anti-Lewis (Le) monoclonal antibodies (Mabs). Using an ELISA technique *H. pylori* strains were screened using a panel of Mabs against Le and other related blood group antigens. Subsequently, LPSs from thre non typeable strains were extracted using the hot phenol-water technique and analyzed electrophorectically (SDS-PAGE) to confirm expression of an O-side chain. The carbohydrate portions of the LPSs were liberated by mild acid hydrolysis and, subsequently, studied by sugar and methylation analyses, 1H-NMR spectroscopy, and electrospray ionization mass spectrometry. The O- side chains of the three Danish *H. pylori* isolates were a polymer of a trisaccharide repeating unit composed of 3-*C*-methyl-D-Mannose (D-Man3Cme), L-rhamnose, and D-rhamnose. These were distinguished from previous strains by their lack of fucosylation, absence of Le antigens and production of an unique O-side chain. Thus these studies indicate that some *H. pylori* strains can produce O-side chains not resembling Le antigens and the occurrence of D-Man3Cme is noteworthy as this sugar has not hitherto been found in nature. The relevance of this sugar to *H. pylori* pathogenesis is presently under investigation.

**HP45** **Vacuolating cytotoxic activity of forty *Helicobacter pylori* strain isolated from Turkish patients**

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**Background:** *H.pylori* (Hp) is a causative agent of gastritis, peptic ulcer and gastric cancer. The *Hp* VacA toxin plays a major role in the gastric pathologies associated with this bacterium. When added to cultured cells, VacA induces vacuolation. The aim of this study was to investigate the in vitro cytotoxin activity of *Hp*. **Materials and Methods:** *Hp* isolates were obtained from patients undergoing upper gastrointestinal endoscopy at the Gastroenterology Clinics of University of Ankara Ibn-I Sina Hospital. A total of 40 strains, 20 from patients with gastritis, 11 from patients with gastric ulcer and 9 were from duodenal ulcer patients, were tested for their vacuolating activity, using Vero cells. After primary isolation, the isolates were grown in brain-heart-infusion broth containing 10 % fetal calf serum (FCS), for 72 h at 37° in microaerobic conditions. Broth cultures were centrifuged at 4°C at 7000 rpm for 15 minutes; the supernatants were filtered and stored at –20°C until tested for vacuolating toxin activity. Vero cells were cultured in tissue culture plates in Dulbecco modified Eagle's medium with 5 % FCS in 5 % $CO_2$ atmosphere at 37°C. After 24 hours, the samples of the culture broth supernatants in triplicate were added to cells at dilutions 1:2 up to 1:32. After further incubation for 24 h, cells were examined microscopically for vacuolization. Tests were scored as positive if at least 50 % of the cells were vacuolated at any dilution. **Results & Discussion:** Of the 40 *Hp* strains 24 (12 from gastritis, 6 from gastric ulcer, 6 from duodenal ulcer patients) were cytotoxic for Vero cell line. Vero cells showed cytotoxic effect in 60 % of the strains examined. Cytotoxic activity seen in Vero cells were detected at 1:4 and 1:8 dilutions. There was no significant difference of *Hp* strains with positive vacuolating cytotoxin activity among patients with peptic ulcers and gastritis.

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**HP46** **Evidence Supporting a Post-Translational Modification of the *Helicobacter pylori* Catalase (KatA)**

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A distinctive feature of the physiology of *H. pylori* is it's extremely active catalase (KatA). The mass of KatA has been determined by SDS-PAGE to have a mass of 50-54 kDa. This is considerably less than the predicted mass, inferred by the gene sequence. The aim of this investigation was to explain the mass difference observed. KatA was purified by medium pressure liquid chromatography. The molecular mass of the purified KatA was determined by electrospray ionisation mass spectrometry (ESI) and was found to be 56.98 kDa, 1.7 kDa smaller (approx.14 amino acids) than the predicted mass (58.69 kDa), as inferred from the gene sequence. Size differences of this magnitude are generally associated with cleaved *N*-terminal signal peptides, occurring during the translocation of proteins. The *N*-terminal sequence of the purified catalase was found to be identical to the predicted amino acid sequence, establishing that there was no *N*-terminal cleavage. This suggested that some other form other post translational modification is occurring. Internal splicing of proteins in prokaryotes appear to be isolated to the acid fact bacteria and the thermophillic archeae. The nucleotide sequence of *katA* was analysed and no regions that corresponded to internal cleavage motifs of spliced proteins were identified, indicating that the cleavage of the protein may occur at the *C*-terminus. The function of this apparent cleavage is yet to be defined. Sequence data has also allowed us to identify a series of interesting motifs at the *C*-terminus of the protein, including a putative amphipathic helix and tetra lysine motif. Current models of protein secretion pathways do not include cleaved *C*-terminal peptide sequences. Based on experimental data we hypothesise that the *C* -terminus of KatA is involved in the translocation of KatA from the cytoplasm into the periplasm and as a consequence the *C*-terminus of the native protein is cleaved.

## *cagE* is essential for gastric colonisation in the Sydney Strain mouse model HP47

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**Background:** The *cag* pathogenicity island (*cag*PAI) of *H. pylori* includes a number of genes involved in a secretory system that may be responsible for the export of virulence proteins. *cagE* within the *cag*PAI is a probable component of the secretion apparatus and is required for induction of IL-8 from gastric epithelial cells. **Objectives:** To determine the importance of *cagE* in the colonisation of the gastric mucosa of mice. **Methods:** An *H. pylori* isogenic mutant was constructed in the Sydney Strain (SS1) by insertional inactivation of the *cagE* gene with a kanamycin cassette. The mutant was confirmed by PCR and restriction enzyme analysis. C57BL/6j ($n$=93) and BALB/c ($n$=93) mice were orally infected with SS1, the *cagE* mutant or sterile broth as the control. Mice were sacrificed at 4, 12 and 24 weeks post infection and stomachs assessed for colonisation. Colonisation was assessed by bacterial counts and histological analysis at 1, 4, 8 and 12 weeks and by the urease assay at 24 weeks Sections from the antrum (A), antrum/body transitional zone (ABTZ), body (B), body/cardia TZ and cardia (BC/C) were graded on a 0-4 scale. **Results:** Although the *cagE* mutant grew normally *in vitro*, when administered into C57BL/6j and BALB/c mice it failed to colonise at every time point. In contrast, the parental SS1 strain colonised 100% of mice with the level of colonisation in BALB/c being slightly lower than that in C57BL/6j mice, a trend we have observed previously. **Conclusion:** *cagE* is a critical determinant for colonisation of the mouse gastric mucosa. This is a surprising and interesting finding given that *H. pylori* strains without the *cag*PAI are able to colonise the human gastric mucosa although perhaps at a different location from *cag*PAI positive strains.

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## A *nixA* mutant of *H. pylori* Sydney Strain (SS1) with reduced urease activity still colonises mice but has different colonisation patterns. HP48

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**Background:** *H. pylori* mutants completely devoid of urease activity fail to colonise the gastric mucosa. However, whether a strain with intermediate urease activity can colonise mice has not been tested. The *nixA* gene encodes a nickel transporter that transports nickel ions for incorporation into apo-urease. Isogenic *nixA* mutants of other strains of *H. pylori* have reduced urease activity. **Objectives:** 1) To compare *in vitro*, the urease activities of the wild type SS1 and a *nixA* mutant. 2) To define and quantitate the colonisation patterns of the mutant as compared to SS1. **Methods:** The *nixA* mutant was constructed by disruption of the *nixA* gene with a kanamycin cassette and confirmed by Southern blotting. C57BL/6j ($n$=113) and BALB/c ($n$=113) mice were infected with ~$10^8$ of either SS1 or the *nixA* mutant or sterile broth (negative control). Bacterial counts and histological analyses were used to assess colonisation at 4, 12 and 24 weeks. Sections from the antrum (A), antrum/body transitional zone (ABTZ), body (B), body/cardia TZ and cardia (BC/C) were graded on a 0-4 scale. **Results:** The *nixA* mutant had 50% decreased urease activity *in vitro* (22,447 nmol of $NH_3$/min/mg protein [units], $n$=10), versus SS1 (45,704 units, $n$=5). Culture results showed that colonisation by the mutant in BALB/c mice was moderately lower overall as compared to SS1, while levels in C57BL/6j mice were similar for both strains. The preferred sites of colonisation in C57BL/6j mice for both SS1 and the mutant were the A and ABTZ. In BALB/c mice, the preferred sites were the ABTZ and BC/C (SS1) or ABTZ (mutant). In relation to patterns of colonisation, lower grades of colonisation were seen for the mutant in the B (C57BL/6j) and the BC/C (BALB/c) as compared to SS1, Colonisation levels in the B of C57BL/6j mice by both SS1 and the mutant were found to increase over time. **Conclusion:** Subtle differences in the patterns of colonisation were observed confirming the role of urease in determining the localisation of gastric infection.

HP49

Characterization of the Outer-Membrane Proteins of a Murine Pathogen, *Helicobacter bilis*

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*Helicobacter bilis* is a bacterial pathogen associated with multifocal hepatitis and inflammatory bowel disease in certain strains of mice. This bacterium was isolated from the liver, bile and lower intestine of mice and is also isolated in other animals such as rats, gerbils and dogs. The gastric pathogen *Helicobacter pylori* has a unique outer membrane proteins (OMP) family, some members of which act as porins (involved in permeability) and adhesins. In this study, the outer membrane protein profiles of four *H. bilis* isolates from mice, dog, rat and gerbil were characterized and compared with that of *H. pylori*. These *H. bilis* isolates displayed similar OMP profiles distinct from that of *H. pylori*. Immunoblotting demonstrated that OMPs between *H. bilis* and *H. pylori* share little cross-antigenicity except for their flagellins. By using two-dimensional SDS-PAGE, five heat-modifiable (HM)-OMPs with the respective molecular masses of 87-, 68-, 52-, 46-, and 35-kDa were identified. The N-terminal sequences of the 35-, 46- and 87-kDa HM-OMPs have no homology with protein sequences available in public databases. These results indicate that *H. bilis* isolates contain a conserved, unique OMP profile that may contribute to its survival in the bile and liver.

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HP50

**Experimental gastric infections on C57BL/6Ncrj mice with fresh strains of *Helicobacter pylori* isolated from patients with gastrointestinal diseases**

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**We studied the abilities in colonization of 58 strains of *Helicobacter pylori* isolated from 58 patients with various gastrointestinal diseases (gastric carcinoma : 8, MALToma : 3, gastric ulcer : 21, duodenalulcer : 15, gastoroduodenal ulcer : 5 and gastritis : 6 ) on mice (SPF, C57BL/6Ncrj strain, 4 weeks old, same number of male and female). Each mouse was challenged orally and only once with 0.5ml of the bacterial growth contained $10^8$-$10^9$ CFU/ml. In the first experiment, the infections abilities of 58 strains on 16 mice each were assessed histopathologically and bacteriologically at 6, 12 days after the bacterial inoculations. In the second, 5 strains which seemed more infectious in the first trial, were challenged again and assessed at 5, 10 and 20 weeks after the inoculations.**

**The strains isolated from gastroduodenal ulcers tended to cause histrogical changes more severely than the strains from the others. One of the gastroduodenal strains was bacteriologically recovered from the stomach at 5 and 10 weeks, and caused severe infiltlation of neutrophillic leukocytes at 5 and 10 weeks, and hyperplasia and dysplasia of glandular cells at 20 weeks.**

## Characterization of *Helicobacter pylori* α1,2 Fucosyltransferase, a Key Enzyme Involved in the Synthesis of Lewis Antigens

HP51

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*Helicobacter pylori* lipopolysaccharides express complex carbohydrates known as Lewis antigens which may contribute to the pathogenesis and adaptation of the bacterium. Involved in the biosynthesis of Lewis antigens are α1,3/4 and α1,2 fucosyltransferases (FucTs). In this study, the Hp α1,2 *fucT* gene was overexpressed in *E. coli*, from which its enzymatic activity was determined. Compared to the Hp α1,3 FucT identified previously, Hp α1,2 FucT has a lower level of expression and the enzyme is rather unstable. A series of substrate (acceptors) were chosen to examine the acceptor specificity of Hp α1,2 FucT in comparison with mammalian α1,2 FucTs, and the enzyme reaction products were identified by capillary electrophoresis. In contrast to the normal mammalian α1,2 FucT (H or Se enzyme), Hp α1,2 FucT prefers to use Lewis X [βGal 1-4 (α Fuc1-3) βGlcNAc] rather than LacNAc [βGal 1-4 βGlcNAc] as a substrate, suggesting that *H. pylori* uses a novel pathway (via Lewis X) to synthesize Lewis Y. Hp α1,2 FucT also acts on type 1 acceptor [βGal 1-3 βGlcNAc] and Lewis a [βGal 1-3 (α Fuc1-4) βGlcNAc] to synthesize H type 1 and Lewis b epitopes. This is the first bacterial α1,2 fucosyltransferase that has been characterized.

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## Detection of helicobacter-like organisms in the abomasum of sheep

HP52

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Since the isolation of *Helicobacter pylori* in humans, several host-specific helicobacters have been described in animals. Helicobacter-like organisms have not been reported in the abomasum of sheep so far. In this study, the presence of helicobacter-like organisms (HLO) in the abomasum of sheep was studied. Twenty-one abomasal stomachs from healthy slaughterhouse sheep, originating from different farms, were examined. Samples were taken from the pyloric region for urease testing, immuhistochemistry and PCR. Fifteen stomachs (15/20) were found positive with the urease test. Immunohistochemical evaluation using a polyclonal antibody raised against *Helicobacter pylori* revealed the presence of HLO in 19 stomachs (19/21). Different morphological types could be distinguished. Clusters of short spiral and coccoid – like bacteria were seen in the gastric mucus layer and the gastric crypts of 19 stomachs (19/21). In three stomachs (3/21) tightly coiled gastrospirillum-like bacteria were observed laying separately in the gastric crypts. It is not clear whether these organisms represent multiple bacterial species. PCR was performed on all stomach samples using two different primer sets. In a first stage, 19 stomachs (19/21) were found positive for the presence of *Helicobacter* species using genus-specific primers. All PCR–positive stomachs were confirmed positive by immunohistochemistry. In a second stage a *H. bovis* – specific PCR assay was used to determine the presence of bovine helicobacters. All samples reacted negative. Further 16S rDNA – analysis will have to determine whether the HLO observed in this study actually belong to the genus *Helicobacter* and represent new species specific for sheep or not.

## HP53 "Candidatus *Helicobacter suis*", a gastric helicobacter from pigs and its phylogenetic relatedness to other gastrospirilla

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"*Gastrospirillum suis*" is an uncultured tightly spiral micro-organism that has been associated with ulcer disease in the stomach of pigs. In this study the phylogenetic position of "*Gastrospirillum suis*" was determined. Stomachs of five "*Gastrospirillum suis*" – infected slaughterhouse pigs originating from different Belgian and Dutch farms were selected. Using broad range primers, bacterial 16S rDNA was amplified by PCR and five helicobacter-like sequences were determined either by direct or indirect sequence analysis. An intersequence homology of 99.7 % was observed, suggesting that the sequences originated from strains belonging to a single species. Phylogenetic analysis of the consensus sequence placed the organism within the genus *Helicobacter*, where it formed a distinct subgroup together with other gastrospirillum-like bacteria (*H. felis*, *H. bizzozeronii*, *H. salomonis*, "*H. heilmannii*" type 1 and type 2). Diagnostic PCR-primers and a probe were developed, differentiating the porcine sequences from all known helicobacters. These results indicate that the porcine sequences represent a single taxon within the genus *Helicobacter*. The low similarity level towards *H. salomonis* (96.6 %), its closest validly named neighbour, strongly suggests that this novel taxon indeed is a novel *Helicobacter* species. In situ hybridisation experiments linked the reference sequence to the "*Gastrospirillum suis*"-like bacteria. On the basis of these results we propose the name "Candidatus *Helicobacter suis*" for this gastric helicobacter from pigs.

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## HP54 Phylogenetic characterization of "Candidatus *Helicobacter bovis*", a new gastric helicobacter in cattle.

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Recently helicobacter-like organisms have been reported in the pyloric part of the abomasum of calves and adult cattle. Cultivation of these spiral bacteria has not been successful to date. In the present study, we used comparative 16S rDNA sequence analysis to determine the taxonomic position of these bacteria. Seven abomasal biopsies of adult cattle were sampled from different Belgian and Dutch farms. In all samples the presence of helicobacter-like organisms was demonstrated by biochemical, immunohistochemical and electron microscopical data. Bacterial 16S rDNA was amplified by PCR and sequences were determined either by direct or indirect sequence analysis. Pairwise comparisons revealed all sequences to be more than 99 % homologous. Phylogenetic analysis placed the organism, corresponding to the reference sequence R2XA, within the genus *Helicobacter*. A diagnostic PCR-assay was designed, differentiating all of the bovine 16S rDNA sequences from Helicobacter and *Wolinella* species. The low similarity level towards *H. bilis* (92.8 %), its closest validly named neighbour, indicates that this novel taxon indeed is a novel *Helicobacter* species. An in situ hybridisation procedure associated the bovine sequences to the helicobacter-like organisms in the abomasum. We propose the name "Candidatus *Helicobacter bovis*" for this new abomasal *Helicobacter* from cattle.

## VARIATIONS OF *H. PYLORI* DURING EXPERIMENTAL INFECTIONS IN MICE

HP55

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Background and aims. *H. pylori* is highly adapted to the human stomach and the clinical isolates show a high diversity which could be due to adaptative changes of the strains passing from a host to another. The aim of this study was to evaluate the adaptative variations of *H. pylori* strains passing from a human host to a murine host.

Methods. C57BL/6 mice were orally infected with 2 infecting strains (IS) freshly isolated from human. The animals were sacrificed at days 3, 7, 14, 21, 45, 90 and 120. Infection of mice was followed by culture of emerging strains (ES) and PCR. Phenotypic variations between IS and ES were studied by SDS-PAGE of saline and total bacterial extracts, and genotypic variations were studied by RAPD using 4 different primers.

Results. ES were isolated from all the mice except at day 3 for one strain. ES undergo variations which appeared as soon as day 3 and which varied with the strain and the bacterial extract. These variations consist mainly in a modification of expression of 3 proteins of high molecular weight. Their pHi were determined by IEF and partial sequence of the purified molecules showed homologies with 3 *H. pylori* proteins : (1) a DNA directed RNA polymerase homolog, (2) a NADH ubiquinone oxydoreductase, and (3) the IF-2 translation factor. Genotypic analysis of all the IS and ES by RAPD showed major modifications of the genome structure at day 3 and minor modifications from days 7 to 45.

Conclusions. The adaptation of a *H. pylori* strain to a new *in vivo* environment occurs during the early stages of the mouse infection and consists in phenotypic and genotypic modifications.

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## Characterisation of *H.pylori* Sialic Acid Binding Lectin

HP56

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The attachment of *H. pylori* to the human gastric mucosa and gastric epithelium is a complex process involving several specific structures recognised by cell surface receptors. Various *H. pylori* strains were compared for binding to sialylated glycoconjugates by showing the requirement of the group of the strains for α-2,3-linked sialic acid. Binding of the bacteria to the panel of neoglycoproteins confirmed the specific interaction. The localisation of the sialic acid-binding lectin on the bacterial cell surface was investigated by immuno-electron microscopy. No gold particles were located on the flagellar sheath, whereas a distribution of the receptor over the bacterial membrane was observed. The corresponding sialic acid-specific lectin was purified from water extracts by affinity chromatography and detected by overlay analysis using sialylneoglycoconjugates.

**HP57** C- Reactive protein and proinflammatory cytokines in the pathogenesis of gastroduodenal ulcers and *H pylori* infections .

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Although *Helicobacter pylori* is a non - invasive organism, the infection results in mucosal infilteration of inflammatory cells in the gastric mucosa. The exact mechanism of inflammatory reaction and its role in the pathogenesis of *H pylori* is not clearly understood. In this study we sought an association between the levels of C-reactive protein , proinflammatory cytokines and active chronic gastritis with and without *H pylori* infection. C-reactive protein was detected in 85 of 200 ulcer patients (43%) as compared to 2of 16 normal controls(13%).It was detected more in *H pylori* infected ulcer patients than in chronic gastritis and lymphoid hyperplasia patients. 52% of gastroduodenal ulcer patients without *H pylori* infection and 57% of patients with *H pylori* infection had raised levels of CRP as ( 13.25 –13,86 mg /ml) compared to normal controls <6mg/ml ( $P < 0.005$). The mucosal production of proinflammatory cytokines , IL-1b, IL-6 and TNF a were determined by short term culture of mucosal biopsy specimens. All 3 cytokines tested by Enzyme Linked Immunoabsorbent Assay (ELISA) were found to be significantly increased in *H pylori* infected patients as compared to controls. IL-1b was 328pg/ml in patients and 106 pg/ml in controls ($P<0.001$), IL6 was 186pg/ml in patients and 80pg/ml in normal controls ($P<0.001$), TNF –a was found to be 410pg/ml in patients as compared to 126pg/ml in *H pylori* negative controls($P<0.001$). Increased production of C-reactive protein , TNF-a, IL-6 and IL1b in chronic gastritis associated with *H pylori* infection may be responsible tissue damage and mucosal inflammation and gastroduodenal pathology.

**HP58** CYTOKINE mRNA EXPRESSION DURING TRANSIENT AND PERSISTENT *H. PYLORI* INFECTION IN RHESUS MONKEYS. A. DUBOIS, A. WELCH, T. WIGGINTON, L. JONES, R. KAMPEN, and A. KIRK. Department of Medicine, USUHS and Transplantation Biology Laboratory, NMRC, Bethesda, MD; and Diagnon, Inc, Rockville, MD, USA.

Up to 90% of rhesus monkeys can be persistently infected by *H. pylori* either naturally or following experimental inoculation with strains isolated from humans. However, 10% of socially-housed monkeys appear to resist natural *H. pylori* infection and they develop only transient infection following experimental inoculation (Infect Immun 1996;64:2885-91). To test the hypothesis that this resistance is due to specific differences in T cell- or macrophage-mediated local immune response, we determined the effect of *H. pylori* inoculation on cytokine mRNA expression in gastric mucosal biopsies. $10^9$ CFU of *H. pylori* strains isolated from humans were injected into the stomach of 6 rhesus monkeys, and pinch biopsies were obtained at endoscopies performed 6, 14, 64 and 310 days later. *H. pylori* status was determined by culture, histology and PCR. mRNA expression for IL-1β, TNF-α, IL-8, IL-10, and IFN-γ was determined and normalized to β-actin. By day 310, 2 animals still were persistently infected (PER) whereas the 4 other ones were not. Initially, gastritis was observed in all animals, but it remained present only in PER animals. mRNA expression of the Th-1-inhibitory cytokine IL-10 mRNA increased only in PER animals, and IL-1β, IL-8, and IFN-γ increased earlier in these animals. Thus, local immune responses initially differ in animals with transient vs persistent infection, and the nature of these initial responses may determine the success or failure of *H. pylori* colonization.

NOTES

HV2

Prophylactically immunized mice exhibit a transient *Helicobacter pylori* infection after challenge. CA Garhart, SJ Czinn, and JG Nedrud. Department of Pathology, Case Western Reserve University, Cleveland, OH, USA

Preliminary quantitative culture experiments on C57BL/6 mice immunized with a mixture of cholera toxin and soluble sonicate from *H. pylori* SS2 demonstrated that intranasal administration of antigen produced greater protection from infection at 4 weeks post challenge than gavage administration. To further characterize the kinetics of colonization in immunized and unimmunized mice, groups of intranasally immunized and unimmunized mice were sacrificed at 3 days and 1, 2, 4, and 8 weeks following challenge with $10^7$ colony forming units of *H. pylori* SS2. At 3 days and 1 week after challenge, there was no difference in colonization levels between the immunized and unimmunized mice. Both groups harbored approximately 6.4 log cfu/g stomach. By 2 weeks, 6/6 immunized mice had a greater than 2 log decrease in bacterial load compared to the unimmunized mice (mean log cfu/g±SD of 3.66±0.44 versus 6.72±0.18, respectively). Four of 6 immunized mice were culture negative at 4 weeks post challenge, and 2/6 mice had greater than 2 log decreases in colonization. At 8 weeks, 3/7 immunized mice were culture negative while 4/7 had greater than 3 log decreases in bacterial load. Unimmunized mice remained heavily infected at 4 and 8 weeks with 6.73±0.37 and 6.99±0.08 log cfu/g, respectively. These results indicate that prophylactic immunization does not prevent colonization by *H. pylori*. However, immunized mice are able to clear the infection or significantly reduce the number of colonizing bacteria. Later time points, currently in progress, will determine whether immunized mice remain protected.

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HV3

**Immunization of C57BL/6 Mice with Sonicated *Helicobacter pylori* and CpG Oligonucleotide Induces Specific Mucosal IgA**

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Mucosal delivery of antigens requires an effective adjuvant to induce mucosal immunity. Current mucosal adjuvants include cholera toxin (CT) and *E. coli* heat-labile toxin. In this study, mice were immunized with sonicated *Helicobacter pylori* protein(sHp) and a novel molecular adjuvant, CpG oligonucleotide. Unmethylated CpG motifs have been shown to induce a broad immune response. Previously, sytemic administration of CpG plus antigen has been shown to induce a predominantly Th1 response. We show that mucosal administration of sHp with CpG can induce mucosal immunity. *Helicoabcter spp.* free C57BL/6 mice were assigned to 7 groups. 4 groups received sHp either with CpG and/or CT or without adjuvant intranasally. 1 group received sHP plus CpG subcutaneously and 1 group received CpG intranasally. Another group which received no vaccines served as controls. All experimental groups received vaccines on weeks 0, 4, 8, 12 and 16. Serum IgG and fecal IgA were assayed every other week. All groups receiving sHp plus CpG and/or CT showed increased serum anti-sHp IgG. Serum IgG subtyping showed a slightly Th2 response in all mice immunized with adjuvantation. Mice immunized intranasally with CpG plus sHp showed a specific fecal IgA response, with or without CT. All animals were challenged with $10^7$cfu *H. felis* three times over a five day period by gastric intubation on week 20, and were sacrificed on week 26. Challenge results will be presented at the workshop.

**HV4**

The UreaseB Subunit Shows a High Degree of Conservation Among 10 *Helicobacter pylori* Isolates

SG RIJPKEMA, Z DURRANI, J BYGRAVES, J RUSSELL, B DAVIES*. National Institute for Biological Standards and Control, Potters Bar, and *School of Biological Sciences, University of Birmingham, Birmingham, United Kingdom.

**Aim.** The *ureB* gene of *Helicobacter pylori* has been reported to be extremely variable. We determined if the variability found at the nucleotide (nt) level also occurred in the translated amino acid (aa) sequence. Ten *H. pylori* strains were selected: these represented 5 *ureB* genotypes. Two strains were cagA negative and 8 strains were cagA positive. One strain was urease negative. Two *ureB* sequences already existed (Acc. nrs: HPAE 000529 and A31515). The Wisconsin package version 9.1 was used to compare nt and aa sequences.

**Results.** Overall, 297nt substitutions were found in the *ureB* gene, which had a maximal length of 1739 bp. These mutations resulted in 41 aa substitutions, and of these only three were non-conservative. Most aa substitutions occurred toward the C-terminal end of the protein. In the urease negative strain a frameshift occurred in nt position 162, which resulted in a stop codon at position 240. In 3 other strains the location of the stopcodon was at a position 10-160 nt upstream of the consensus sequence.

**Discussion.** We conclude that most mutations at the nt level are silent, and that the UreB subunit is likely to be highly conserved among *H. pylori* strains. Therefore this antigen remains an important vaccine candidate

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**HV5**

T-cell response of of *H. Pylori* Infected Balb/cA Mice Treated with an Antioxidant (Astaxanthin) in Short Term or Long Term Treatments.

M BENNEDSEN, X WANG[1], R WILLÉN[2] T WADSTRÖM[1] & LP ANDERSEN[1]. Rigshospitalet, Copenhagen Denmark.[1]University of Lund, [2]University of Gothenburg, Sweden

**Introduction:** Treatment of mice with anstaxanthin reduces bacterial load and gastric inflammation of infected Balb/cA mice. Low intake of carotenoids and vitamine C has been proposed as a risk factor for acquering the *H. pylori* infection. **Aim:** To evaluate the cytokine production of splenocytes of *H. pylori* infected mice treated with Astaxanthin versus T-cells from untreated or uninfected mice. **Materials & Methods:** Short term treatment:20 mice were infected with *H. pylori* and 10 mice were uninfected. 2 weeks post-inoculation 10 mice were treated orally with Astaxanthin (200 mg/kg/day) (AstaCarotene, Gustavsberg, Sweden) for 10 days and then all mice were sacrificed. Long term experiment: 16 mice were infected (8 uninfected) for 4 months, then treated for 4 weeks with astaxanthin and then sacrificed. Bacterial load was estimated by culture of gastric mucosa. Isolated splenocytes were stimulated with *H.pylori* sonicate for 36 hours. Culture supernatants were analysed for IFN-g and IL-4 by ELISA. **Results & discussion:** In both short term treatment and long term treatment with astaxanthinsignificant reductions in bacterial load and gastric inflammation as compared to infected, untreated mice were seen ($p<0,05$). This was associated with a tendency towards lower IFN-γ release by the splenocytes. Following the short term treatment regime, IL-4 release of splenocytes were induced in the treated mice, but neither in the uninfected nor in the infected untreated mice. Following the long term treatment regime, no IL-4 was induced in any of the groups. This suggests, that the Astaxanthin induced modulation of the immune respons to *H. pylori* in mice may be transient.

HV6

**Effect of interferon gamma and tumor necrosis factor alpha on the oxidative burst of monocytes when stimulated with *Helicobacter pylori***

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[1]Hacettepe University, Medical Faculty, Ankara, Turkey, [2]Lund University, Medical Faculty, Lund, Sweden, [3]Aalborg Hospital, Aalborg, Denmark [4]Rigshospitalet, Copenhagen, Denmark.

**Introduction:** *H. pylori* is able to activate monocytes in a dose dependent way. The interaction of cytokines on this activation has never been investigated.
**Aim**: To observe the influence of interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α) on the oxidative burst response of monocytes stimulated with *H. pylori* strains from duodenal ulcer disease and gastritis.
**Materials and Methods**: Oxidative burst response was measured by chemiluminescence assay. Two *H. pylori* strains, one from a gastritis patient and one from a duodenal ulcer patient were used to stimulate oxidative burst in phagocytes from five donors (3 male and 2 female). Two sets of experiments were designed: 1) monocytes were primed with *H. pylori* sonicate for 30 minutes, then with IFN-γ for 30 minutes and then stimulated with opsonized zymosan (OZ), 2) monocytes were primed with IFN-γ then with *H. pylori* sonicate and stimulated with OZ. The same procedure was performed for TNF-α.
**Results and Discussion**: The oxidative burst response of the monocytes were not significant according to any of the variables, i.e., there was no significant difference between the responses of the donors, no significant difference could be shown in the two strains, also and there was no significant influence, on the monocytes neither when they had been primed with IFN-γ nor with TNF-α. Thus, TNF-α and IFN-γ do not interact with the oxidative burst response of monocytes.

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HV7

**The IgG Subclass Responses to *Helicobacter pylori* - Markers of Duodenal Ulcer Disease and Inflammation**

*HM MITCHELL, *K MASCORD, #SL HAZELL and &G DASKALOPOULOS
*School of Microbiology and Immunology, University of New South Wales, University of Western Sydney, and & The Inner West Endoscopy Centre, Australia

**Background**: In many viral, bacterial and parasitic infections the IgG subclass response to infection has been shown to correlate with both severity of inflammation and disease outcome. To date, there is little information on the relationship between the IgG subclass response and *H. pylori* infection. **Aim:** To investigate the association between the IgG subclass response to *H. pylori* infection and disease and inflammation in subjects with non-ulcer dyspepsia (NUD) and duodenal ulcer disease (DU). **Methods**: Eighty three symptomatic patients attending a private gastroenterology clinic in Sydney, Australia for investigation of upper gastrointestinal symptoms were included in the study. Upon endoscopic examination, the presence of ulceration was noted and biopsy specimens were collected from the gastric antrum, body and transitional zone. Blood was also collected from each patient. Gastric biopsy sections were graded using the Sydney system. *H. pylori* specific IgG, IgG1, IgG2, IgG3 and IgG4 were measured by ELISA. The IgG subclass was also examined retrospectively in sera collected from 20 patients previously proven to have DU. **Results:** At endoscopic examination, 14 subjects had DU and 9 had evidence of past DU. IgG serology and histological examination showed 35 subjects to be *H. pylori* negative and 48 to be *H. pylori* positive. Of these 48 patients, 25 had NUD and 23 past or present DU. Examination of the IgG subclass response to *H. pylori* showed significantly higher levels of IgG2 antibodies to be present in patients with both past and present DU as compared with patients with NUD ($p=0.009$). In addition, significantly higher IgG3 subclass antibody levels were associated with increased levels of chronic and active inflammation throughout the stomach. This was significant in the case of chronic inflammatory cells in the body ($p=0.028$) and active inflammatory cells in the junctional zone ($p=0.01$). **Conclusion:** This study suggests that the IgG subclass response in subjects infected with *H. pylori* may be a marker of duodenal ulcer disease and increased levels of inflammation.

HV8

**Serologic Response by Western Blot to *Helicobacter pylori* (Hp) in a Cohort of Mexican Children During the First Year of Life.** A. SOLANO, V. LUQUEÑO, Z. L. BARAJAS, M.L. GUERRERO, G. M. RUIZ-PALACIOS. Instituto Nacional de la Nutrición, Mexico City, Mexico.

**Background.** It has been observed that anti-Hp specific antibodies pass the placental barrier and disappear by the age of 3 months. Moreover, children born to Hp-positive mothers do not seem to have an increased risk of becoming actively infected with Hp during the first year of their lives. **Objective**. To evaluate the evolution of the serologic status of infants born to Hp-positive and Hp-negative mothers in a cohort of Mexican infants who live in an area were the prevalence of Hp infection is high. **Methods**. From March 1998 to May 1999, a cohort of 30 mother-infant pairs were followed prospectively from birth to 12 months in a periurban area of Mexico City. Blood samples were drawn at birth and every three months. Specific serum IgG antibodies were detected by Western Blot (WB) analysis using a sonicate antigen. A positive serologic response was considered when at least 2 or more bands between 6.5 and 42 kDa and/or at least one band above the 66 kDa were observed; if a seropostive mother, the child above 6 months of age was considered positive if he/she had the same intensity- positive serologic response as the mother in two consecutive tests; when a new band was observed or if the child had turned negative to the mother seroresponse and the same pattern reappeared. **Results.** Mother-infant pairs were monitored for a total of 312 child-months of observation. At birth, WB was positive in all infants born to seropositive mothers (n=27) and negative in those born to seronegative mothers (n=3). Of the 27 infants with initial positive WB, 12% had turned negative by 3 months of age, 45% by 6 months, 30% by 9 months and 13% by 12 months of age. By either definition, 11 (IR=35 infections/100 child-month) infants had turned positive by WB. Infection was confirmed by the $^{13}$C-Urea breath test. **Conclusions.** IgG antibodies against Hp cross the placental barrier. Children form this community have an increased risk of being infected by Hp at an early age, Household transmission as well as other risk factors should be further studied.

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HV9

## Identification and Evaluation of Two Outer Membrane Proteins from *Helicobacter pylori* as Vaccine Candidates

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Wyeth Lederle Vaccines, West Henrietta, NY 14586

The need for a vaccine against *H. pylori* has increased in recent years with the rise in antibiotic-resistant strains. We have identified two *H. pylori* outer membrane proteins with MW of 75 and 77 kDa using murine monoclonal antibodies against *H. pylori* whole cells. We have shown with a variety of techniques that the proteins are localized on the bacterial surface and are conserved among a number of clinically relevant strain. The vaccine potential of the proteins was evaluated in prophylactic and therapeutic mouse models using *H. pylori* strain SS1. The purified proteins were administered intragastrically (IG) with wild type or attenuated cholera toxin or subcutaneously (SC) adsorbed to aluminum phosphate. Immunizations IG or SC with the protein-adjuvant formulations prior to or after *H. pylori* infection have resulted in a statistically significant reduction of colonization of *H. pylori* strain SS1 in the stomach tissue as determined by quantitative culture. Furthermore, both systemic and mucosal antibodies to the proteins were detected in serum and mucosal samples. The serum antibodies were shown to have bactericidal activity. These data suggest that the two outer membrane proteins are potential prophylactic and therapeutic vaccine candidates against *H. pylori* infection.

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